THE EFFECTS OF FIRE AND DROUGHT ON PLANT-SOIL FEEDBACKS OF A NON-NATIVE INVASIVE GRASS IN SOUTHERN ILLINOIS

BY

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THESIS

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Abstract

Non-native plants can disrupt ecosystem functioning and internally reinforce their dominance over native species by altering soil nutrient availability (i.e. resource-mediated feedbacks) and by modifying fire regimes (i.e. disturbance-mediated feedbacks). While fire and other disturbances are shown to promote further invasions, there is a limited understanding of their effects on invader-driven biogeochemical impacts. In this thesis, I examine how climate-mediated disturbances including fire and drought affect invader impacts on soil carbon (C) and nitrogen (N) cycling. Working in temperate deciduous forests in southern Illinois, I quantified the effects of fire and drought on belowground soil organic matter pools, microbial biomass, extracellular enzyme activities, and soil respiration in stands invaded by the C_4 exotic grass *Microstegium vimineum*. I used a manipulative field experiment conducted across two invaded sites with contrasting fire history to determine the effects of prescribed burning and growing season drought imposed using rainout shelters. I found that invaded plots exposed to repeated burning had lower invasive grass productivity, higher root:shoot ratio, and higher C:N ratio in plant tissues and soil microbial biomass. The results presented here suggest that invasion by grasses like *M. vimineum* may increase the potential for N loss during fire, leading to a progressive depletion of N availability through repeated burning, which may weaken the positive resource-mediated feedbacks initiated by non-native plants. I also found that drought may further contribute to the weakening of invasive plant-soil feedbacks through co-limitation with N. Though climate change is generally predicted to facilitate the spread and establishment of invasive plants in the future, increases in fire and drought frequency may weaken the self-reinforcing feedbacks and ecosystem impacts of non-native invasive plants.

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CHAPTER 1: LITERATURE REVIEW

Introduction to Non-Native Plant Invasions

Plant invasions are an important agent of global environmental change and can disrupt ecosystem structure and functioning across community types (Ehrenfeld 2010, Vila et al. 2011, Ricciardi et al. 2017). Many studies focus on the ecosystem impacts of invasion, suggesting that invasive plants negatively affect native plant diversity (Vila et al. 2006, Powell et al. 2011), increase ecosystem productivity (Peltzer et al. 2010), and alter soil carbon and nutrient cycles (Liao et al. 2008). Non-native invasive plants can also introduce novel traits into an ecosystem in ways that shift ecosystems toward alternative stable states to favor their own persistence through resource- and disturbance- mediated feedbacks (Suding et al. 2004). One such example is *Microstegium vimineum* (Trin.) A. Camus, an exotic invasive grass that is a widespread and persistent invader in the eastern United States.

History of M. vimineum Invasion

Microstegium vimineum is an annual C_4 grass that is heavily invasive in forest understory communities (Winter et al. 1982, Morrison et al. 2007). It is a shade-tolerant species that prospers under moderate light conditions (Winter et al. 1982, Gibson et al. 2002), and quickly spreads along streamsides, roadsides, trails, floodplains, and areas of previously disturbed forest habitat (Gibson et al. 2002, Morrison et al. 2007, Manee et al. 2015). Its seeds germinate early in the spring, and can form dense thickets of meter-tall grass over the course of the growing season (Morrison et al. 2007). *M. vimineum* was originally introduced into the Eastern United States through its use as a packing material in shipments from Asia throughout the early twentieth century (Barden 1987) and was first observed growing in the United States near Knoxville,

Tennessee in 1919 (Fairbrothers and Gray 1972). *M. vimineum* dominance within temperate forest understories in the Eastern United States has since grown tremendously. By 1972, the invasion had spread to 14 states ranging from Florida to New Jersey, and westward toward Ohio and Mississippi (Fairbrothers and Gray 1972). As of today, *M. vimineum* ranges across nearly twenty-five different eastern states, with the heaviest invasion occurring across land east of the Mississippi River (Culley et al. 2016).

M. vimineum is shown to perpetuate its dominance over native plants by establishing resource-mediated and disturbance-mediated feedbacks. Specifically, *M. vimineum* does this by altering soil N cycles through positive associations with ammonia-oxidizing communities (Shannon-Firestone et al. 2015), increased rates of nitrification (Ehrenfeld et al. 2001, Kourtev et al. 2003, Lee et al. 2012), and greater partitioning of N aboveground compared to native species (Fraterrigo et al. 2011). This alteration in belowground biogeochemical cycling allows *M. vimineum* to establish favorable resource conditions that promote its own productivity. This species can also establish positive disturbance-mediated feedbacks through fire. By increasing ecosystem flammability, *M. vimineum* establishes a positive grass-fire cycle that increases its abundance in comparison to other plants (Glasgow and Matlack 2007, Flory et al. 2015, Wagner and Fraterrigo 2015).

Soil Carbon and Nitrogen Cycling and Resource-Mediated Feedbacks

The impacts of non-native plant invasions and on soil C and N cycling are important because soil organic matter (SOM) represents the largest terrestrial sink of C and stores more than three times as much C as terrestrial vegetation (Schmidt et al. 2011). Perturbations to belowground functioning that speed up decomposition of SOM and reduce the size of the soil C

sink increase the amount of C that is respired from the soil and released to the atmosphere as CO_2. The quantity and quality of inputs to the soil (i.e. litter and plant C:N ratios) combined with ambient nutrient availability closely mediate the microbial carbon use efficiency (CUE; defined as the ratio of C allocated for growth over C respired), where CUE declines with decreasing nutrient availability and increasing substrate C:N ratios (Manzoni et al. 2012). This decline in CUE corresponds with increased heterotrophic respiration and loss of soil C under nutrient limited conditions (Craine et al. 2007). Similarly to CUE, microbial communities can also regulate their nitrogen use efficiency (NUE; defined as the ratio of N allocated for growth over N released in mineralized form, primarily ammonium), where lower substrate C:N ratios and higher N availability overall drives a lower NUE (Mooshammer et al. 2014).

Invasive plants can shift belowground conditions that influence microbial CUE and NUE by producing an excess of litter that has differing chemical properties than native plant litter (Ehrenfeld 2010, Tamura and Tharayil 2014). In consequence, many non-native plant invasions are shown to accelerate rates of soil C turnover (Jackson et al. 2002, Litton et al. 2008, Martin et al. 2009, Koteen et al. 2011), leading to a net release of C into the atmosphere that would have otherwise been sequestered in the soil. Yet other plant invasions increase accumulation of soil C (Hibbard et al. 2001, Wolkovich et al. 2010), as a result of short-term increases in net primary productivity (NPP) associated with invasion (Lett et al. 2004, Wilsey and Polley 2006). Meta analyses have shown effects of invasion on soil N to be similarly mixed (Vila et al. 2011), though many invasive plants are able to outcompete native species through enhanced long-term N acquisition, which drives an increased N flux through the ecosystem (Liao et al. 2008, Castro-Diez et al. 2014) and depletion of soil N concentrations over time (Jo et al. 2015).

Fire as a Disturbance-Mediated Feedback

Fire is an important process for maintaining vegetation structure (Bond et al. 2005), reducing accumulation of fine fuels (Williams et al. 2012), and promoting regeneration of native fire-tolerant species (Abrams 2005). The benefits of fire have led to an expansion of the use of prescribed burning in woodlands across the United States, particularly in forests where non-native plants are abundant (Blake and Schuette 2000, Gilbert et al. 2003, Brose et al. 2013, Ryan et al. 2013). However, the introduction of more frequent fires on landscapes can lead to shifts in plant composition and soil physicochemical conditions that may further promote the persistence of invasive plants and their disturbance to ecosystem functioning (Kuppinger et al. 2010, Matlack 2013).

Despite its widespread ecological benefits, fire can also lead to adverse changes in Eastern deciduous forest communities because of its positive interaction with non-native grass species (Matlack 2013). This positive feedback cycle is set in motion when non-native grass species invade a landscape and alter fire regime properties while recovering from fire at a faster rate than native species (D'Antonio and Vitousek 1992). An invasive grass can impact a landscape's fire regime by increasing fine fuel loads leading to increased fire frequency, intensity, and extent. When regime changes encourage the dominance of invasive plants, the abundance and diversity of native species can decline as a result (Brooks et al. 2004). Positive feedbacks between fire and non-native invasive grasses are consistently observed across species and landscape types (Lesica and Martin 2003, Brooks et al. 2004, Glasgow and Matlack 2007). Fire can also magnify the per capita effects of invasive plants by altering physicochemical conditions, where post-fire changes in soil moisture and nitrogen availability facilitate the performance of non-native grasses after a fire (Craig et al. 2015, Wagner and Fraterrigo 2015).

Climate Change, Drought, and Invasive Plants

Climate change is generally predicted to facilitate the spread and performance of invasive plants in the future (Weltzin et al. 2003, Bradley et al. 2010, Liu et al. 2017). Changes in precipitation, temperature, and CO_2 concentrations can directly impact plants, including invasives, by altering their physiology and traits (Sorte et al. 2013). It has been hypothesized that these climatic changes may promote invasive plant performance relative to natives because non-natives exhibit broader tolerance to altered environmental conditions (Davidson et al. 2011). While invasive plants are capable of altering disturbance regimes (D'Antonio and Vitousek 1992), hydrologic cycles (Wilcox and Thurow 2006), and soil biogeochemical cycles (Mack et al. 2001, Allison and Vitousek 2004) to reinforce their own dominance, the impacts of climate change on these invasion-mediated feedbacks still remain understudied.

Conclusions

Although separately resource- and disturbance-mediated feedbacks can stabilize alternative ecosystem states and impede restoration (Suding et al. 2004, Eviner and Hawkes 2012), their combined effects and potential impacts on each other remain unclear. Studies have focused extensively on the impacts of invasive plants on biodiversity, ecosystem functioning, and soil carbon and nutrient cycles, yet there is limited understanding about how disturbance, such as altered fire regimes and climate change, will alter invasive plant feedbacks over time.

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CHAPTER 2: FREQUENT FIRE MITIGATES THE ECOSYSTEM IMPACTS OF AN EXOTIC GRASS BY WEAKENING PLANT-SOIL FEEDBACKS

Abstract

Fire activity is increasing in many regions due to climate change, land management practices, and changes in fuels associated with non-native plant invasions. While positive feedbacks between fire and invasion are well documented, there is limited understanding of how increased fire frequency will affect the dynamics and impacts of plant invasions, especially where resource-based plant-soil feedbacks underpin invasive plant dominance and altered ecosystem functioning. I measured fire effects on the production, stoichiometry, and biogeochemical impacts of an invasive annual grass, *Microstegium vimineum*, in temperature deciduous forests with a history of either (1) frequent fire or (2) fire exclusion. Compared to invaded stands with a history of fire exclusion, stands subjected to frequent fire were less sensitive to fire and exhibited weaker plant-soil feedbacks, evidenced by lower *M. vimineum* biomass production, and higher root:shoot and tissue C:N ratios. Effects on biogeochemical cycling were also diminished, with fire decreasing soil CO_2 efflux and particulate organic matter in areas of fire exclusion and increasing stocks of organic C and N in plots subjected to frequent fire. Higher microbial biomass C:N ratio and greater activity of the N-degrading enzyme β-N-acetylglucosaminidase suggest that frequent fire can progressively deplete soil N availability in invaded stands. Increased fire frequency may thus reduce invasion impacts on biogeochemical cycling by increasing belowground N-limitation, thereby weakening plant-soil feedbacks over time.

Introduction

Non-native plant invasions that introduce novel traits or shift the abundance of functional traits in a community can have profound effects on ecosystem functioning (Vila et al. 2011, Pysek et al. 2012), and may initiate internal feedbacks that stabilize alternative ecosystem states (Suding et al. 2004). For example, invasions by species that differ from residents in resource acquisition, allocation, and/or release strategy have been shown to alter biogeochemical cycling (Ehrenfeld 2003, Castro-Diez et al. 2014). Such alterations can benefit invasive plants by establishing soil conditions that promote or maintain their dominance, resulting in a positive resource-based plant-soil feedback (Bever et al. 1997, Ehrenfeld et al. 2005, van der Putten et al. 2013). Because internal feedbacks make it difficult to restore invaded systems (Eviner and Hawkes 2012), there is support for prioritizing the management of invasive species that drive ecosystem-level change through internal feedbacks (Gaertner et al. 2014).

However, resource-based feedbacks and their potential impacts on ecosystem functioning over time remain largely unexplored, especially in the context of increasing fire activity. Fire activity is increasing in many regions due to climate change, land management practices such as prescriptive burning, and plant invasions that enhance ecosystem flammability (Moritz et al. 2012, Balch et al. 2013, Ryan et al. 2013). While many studies demonstrate that fire can promote further invasion in part by releasing nutrients (Johnson et al. 2011, Wagner and Fraterrigo 2015), it is unclear how frequent fire affects plant invasions and their associated biogeochemical impacts. Given the rapid expansion of invasive plants and continued increase in fire activity, understanding how frequent fire affects resource-based plant-soil feedbacks is needed to guide management and inform invasion theory.

Resource-based plant-soil feedbacks are thought to be driven by changes in the supply of carbon (C) and nitrogen (N), to soil decomposers (Wolkovich et al. 2009, Inderjit and van der Putten 2010, Zhang et al. 2019). Compared to natives, non-native invasive species have high growth rates (Blumenthal 2005), high resource-use efficiencies (Funk and Vitousek 2007, Heberling and Fridley 2013), and high capacities for soil nutrient acquisition (Jo et al. 2015), leading to increases in the quantity and quality of detrital inputs (Ehrenfeld 2003, Liao et al. 2008, Castro-Diez et al. 2014). They can also produce root exudates, thereby increasing the supply of labile C in the rhizosphere (Bradford et al. 2012). Such changes stimulate the metabolic activities of microbial decomposers, resulting in accelerated organic matter turnover and increased inorganic nutrient supply to plants (Allison and Vitousek 2004, Craig and Fraterrigo 2017).

This microbial response to changes in resource supply is consistent with theoretical and empirical studies demonstrating that microbes adjust their activities to achieve stoichiometric homeostasis in the face of variations in the composition and availability of resources in their environment (Anderson et al. 2005, Frost et al. 2005). Relative to the stoichiometry of the substrates used by microbes, the stoichiometry of soil microbial biomass is significantly more constrained in range and variance, resulting in large stoichiometric imbalances between microbes and their substrates (Cleveland and Liptzin 2007, Mooshammer et al. 2014b). Consequently, microbes must adapt to maintain a near constant biomass C: nutrient ratio, i.e. stoichiometric homeostasis (Sterner and Elser 2002). Microbial decomposers do so mainly by mineralizing and excreting resources in excess of their demand through the regulation of their resource-use efficiencies, especially C-use efficiency (CUE) and N-use efficiency (NUE) (Schimel and Weintraub 2003, Mooshammer et al. 2014b, Manzoni et al. 2017). CUE is defined as the ratio of

the C invested in growth (new biomass production) over total organic C taken up (del Giorgio and Cole 1998), and decreases with increasing substrate C:N ratio, reflecting that microbial growth is more N than energy limited (Devevre and Horwath 2000, Manzoni et al. 2008, Manzoni et al. 2012). Similarly, NUE is defined as the ratio of N invested in growth over total organic N taken up, and decreases with decreasing substrate C:N ratio, reflecting that microbial growth is more energy than N limited (Mooshammer et al. 2014a). When decomposers are N limited, changes in ambient N availability can also affect resource-use efficiencies, with CUE increasing and NUE decreasing in response to increased N availability (Ziegler and Billings 2011, Manzoni et al. 2012, Mooshammer et al. 2014a).

Changes in decomposer resource-use efficiencies strongly affect biogeochemical cycling. For example, a low CUE indicates a smaller amount of C will remain in the soil as microbial biomass and byproducts and a larger amount will be released as CO_2; this reduces the potential for C storage because microbial residues are a precursor to soil organic matter (SOM) formation (Six et al. 2006, Manzoni et al. 2012, Miltner et al. 2012). A low NUE indicates a smaller amount of N will remain in the soil as microbial biomass and byproducts and a larger amount will be mineralized to ammonium, setting the stage for future transformations and loss pathways (Mooshammer et al. 2014a). Shifts in decomposer resource-use efficiencies may thus underpin not only resource-based plant-soil feedbacks but also the ecosystem impacts they engender. Consistent with this hypothesis, invasions that increase substrate C:N ratio (e.g. via exudation) have been shown to increase heterotrophic respiration while reducing microbial biomass and soil C storage (Strickland et al. 2010), while invasions that decrease substrate C:N ratio commonly increase N mineralization and nitrate availability, as well as increase the abundance of microbial nitrifiers (Hawkes et al. 2005, Shannon-Firestone et al. 2015, Morris et al. 2016). Additionally,

changes in ambient N availability have been shown to mediate the ecosystem impacts of the same invader (Monaco et al. 2003). For example, Craig et al. (2015) found that grass-invaded forests with higher ambient N availability had higher soil C stocks, consistent with higher CUE, whereas invaded forests with lower ambient N availability had lower soil C stocks, consistent with lower CUE.

Coordination among plant resource inputs, microbial metabolism, and plant nutrient uptake should favor the persistence of resource-based plant-soil feedbacks. However, a gradual weakening of feedbacks may result from mismatches in the timing of microbial release of nutrients and plant uptake. This could lead to less robust invasive plant populations as well as diminished biogeochemical impacts over time. In seasonally dry Hawaiian woodlands for example, plant-soil feedbacks between an invasive C_4 grass and soil N cycling shifted from positive to negative over two decades, resulting in decreased dominance of the invasive grass. This shift was attributed to greater N losses in invaded forests, which ultimately lowered ecosystem N supply rates (Yelenik and D'Antonio 2013). Slow and cumulative declines in soil resource conditions may thus contribute to long-term changes in the ecosystem effects of invasion (Strayer et al. 2006) as well as boom-bust dynamics of invasive species populations (Strayer et al. 2017).

Fire is known to provide a temporary pulse of N that stimulates plant productivity and N uptake (Christensen 1973, Monaco et al. 2003). However, N contained in biomass and soil organic matter can be mobilized or volatilized and lost from the system under frequent burning, leading to long-term declines in N capital (Binkley et al. 1992, Johnson and Matchett 2001). These losses may scale with the N content of fuels, resulting in proportionally greater N losses in ecosystems with high leaf litter N (Gray and Dighton 2006). Indeed, meta-analysis shows that

repeated fire can strongly deplete soil N, with larger effects observed in ecosystems with higher litter N content (Pellegrini et al. 2018). If invasive plants store proportionally larger amounts of N in aboveground biomass, the effects of fire on N capital could be compounded. Frequent fire in invaded systems may thus accelerate the weakening of resource-based plant-soil feedbacks by increasing nutrient release in the absence of a corresponding increase in plant nutrient uptake, leading to progressive N deficiency. Frequent fire may also drive lower microbial CUE and higher NUE, which would reduce microbial turnover of organic matter and increase soil C and N stocks and the C:N ratio of soil organic matter.

In this study, I investigated the ecosystem-level effects of resource-based plant-soil feedbacks in grass-invaded forests with contrasting fire frequencies. Grass invasions are common, occurring on almost every continent, and can lead to resource-based and fire-mediated feedbacks that stabilize grass dominance (D'Antonio and Vitousek 1992). I focused on the invasive *Microstegium vimineum* (Trin.) A. Camus, a widespread invasive annual C_4 grass that has previously been shown to perpetuate its dominance over native plants by establishing resource-based (Ehrenfeld et al. 2001, Lee et al. 2012, Craig and Fraterrigo 2017) and fire-mediated feedbacks (Glasgow and Matlack 2007, Flory et al. 2015, Wagner and Fraterrigo 2015). Although this species is well studied, it is unknown how frequent fire affects resource-based plant-soil feedbacks and biogeochemical cycling over time. Previous research shows that *M. vimineum* is a strong competitor for N, a majority of which it allocates to aboveground tissues (Fraterrigo et al. 2011). This in turn contributes to intensified plant-microbial competition for N and accelerated C and N cycling, which can be modulated by external N inputs (Craig et al. 2015, Craig and Fraterrigo 2017). At the same time, *M. vimineum* increases fire intensity in closed-canopy forests (Wagner and Fraterrigo 2015), which should lead to greater potential for N

loss. I therefore hypothesized that frequent fire would weaken resource-based plant-soil feedbacks and diminish the ecosystem effects of invasion by causing progressive N deficiency, resulting in decreased biomass production and higher C:N ratios in plant biomass and SOM. To test this hypothesis, I compared the response of invasive plant populations, soil microbial activities and soil chemistry to one-time, experimentally imposed fires in invaded plots across forest stands with a history of either frequent fire or fire exclusion.

Methods

Site Description and Experimental Design

This study was conducted in the Central Hardwood Region of southern Illinois, USA, which has been heavily invaded by *M. vimineum* for more than two decades. I selected two sites for their contrasting fire frequencies: Dixon Springs State Park (DSSP), and Giant City State Park (GCSP) (Appendix A). DSSP (324 ha) is located at the southeastern edge of the Shawnee National Forest (37°22' N, 88°39' W) and was first invaded with *M. vimineum* in the mid-1990s. Since the early 1990s, DSSP has been consistently managed with prescribed fire, which is applied every 3-6 years within the park's woodlands to reduce fine fuel accumulation, promote oak-hickory regeneration, and stimulate the growth of native understory vegetation. GCSP (1,619 ha) is located ~65 km northwest of DSSP (37°36' N, 89°11' W) and was invaded in the early 2000s (Illinois Department of Natural Resources). Prior to the spring of 2016, woodlands in GCSP did not experience fire.

Regional climate is classified as humid subtropical, with hot, humid summers and moderate winters. Mean temperature (since 2005) is 24°C in the summer (June-Aug) and 2°C in the winter (Dec-Feb) for both sites; mean annual precipitation is 135 cm and 122 cm for DSSP

and GCSP, respectively. During the study year of 2017, annual precipitation totals were below average, summing to 114 cm in DSSP and 102 cm in GCSP (http://raws.dri.edu). Soils within the study area are Alfisols (mesic Oxyaquic Fragiudalds) in the Grantsburg series (DSSP) and Hosmer series (GCSP). Soil texture for both series is classified as a silt loam with ~15-25% clay in the 0-15 cm depth (https://websoilsurvey.nrcs.usda.gov). DSSP and GCSP have similar hardwood overstories, containing a mix of predominantly elm (*Ulmus sp.*), maple (*Acer sp.*), ash (*Fraxinus sp.*), black walnut (*Juglans nigra*), shingle oak (*Quercus imbricaria*), sycamore (*Platanus occidentalis*), and sassafras (*Sassafras albidum*) (Appendix B). Both sites have similar elevations (150-200 m) and occur on mild slopes of 2-18%.

In early February 2017, I located well-established *M. vimineum* populations at each site and installed 20 pairs of 5 m x 5 m plots (n = 40 plots total) within the invaded areas. Fourteen pairs were installed at DSSP and six pairs were installed at GCSP. Within each pair, plots were spaced 2 m apart to minimize environmental differences and were randomly assigned to either burn or ambient treatments. Burn plots at each site were treated with fire in early April 2017 using drip torches and a handline surrounding the plot boundary. All 20 burn plots were burned as consistently as possible on the same day. Previous prescribed burn records at DSSP show fire temperatures at the soil surface to range from 75 to 300^{o}C (Wagner and Fraterrigo 2015).

Soil Respiration and Environmental Parameters

In May 2017, I installed two permanent 10-cm PVC collars in each plot. Soil respiration was measured twice monthly in each plot from June – November 2017 with a LI-COR 8100A portable infrared gas analyzer (LI-COR, Inc., Lincoln, NE). All flux measurements were taken during the day between 10:00 and 16:00 hours. Prior to taking measurements, any new plants

that had sprouted within the collar were removed, but litter in the collar was left intact. Concurrently, soil temperature (10-cm depth, Thermo Fisher Scientific, Waltham, MA) and volumetric soil moisture (7.5-cm depth, FieldScout TDR 100, Spectrum Technologies, Inc., Aurora, IL) were measured adjacent to each collar. To further characterize site-level differences in environmental conditions, I deployed data loggers (HOBO Micro Station H21 Data Loggers, Onset Computer Corporation, Bourne, MA) paired with one soil temperature probe (10-cm depth; S-TMB-M002 Temperature Sensor, Onset Computer Corporation, Bourne, MA) and one moisture probe (7.5-cm depth; ECH$_2$O EC-5 Moisture Sensor, Decagon Devices Inc., Pullman, WA) at each site and recorded hourly values from June-November 2017. These data were used to construct a continuous model of soil respiration for each plot (see *Data Analysis* section).

Soil and Vegetation Sampling

Soils were sampled twice throughout the study period: first in July 2017 to determine microbial biomass and enzyme activities, and again in August 2017 to determine C and N content and pH. I collected mineral soil from each plot using a 2.2-cm diameter soil probe to sample from 0-5 cm, 5-10 cm, and 10-15 cm depths. Each sample represented a composite of four individual soil samples taken randomly across the plot. An additional 11 adjacent uninvaded areas were sampled in August to calculate the *M. vimineum* contributions to SOC pools (see *Laboratory Analyses* section). Soils sampled in July were transported to the lab on ice and refrigerated at 4 ^{o}C and immediately assayed for microbial biomass. Subsamples of these soils were frozen at -80 ^{o}C for four months before measuring potential extracellular enzyme activities. Soils from August were air-dried, mixed thoroughly, and sieved (< 2 mm) prior to subsampling for determination of pH, and C and N by combustion for fractionated SOM (see

Laboratory Analyses). Bulk density was determined for each pair using a standard 5-cm bulk density corer and used to calculate C and N density, while adjusting for the mass and volume of any rocks or large roots from the sample.

Roots were sampled at the same time as soils in August 2017. To determine standing root biomass, I collected three replicate cores from each plot (3.5-cm diameter). After separating at 0-5 cm, 5-10 cm, and 10-15 cm depths, samples were composited and then frozen until roots could be processed. Samples were washed with deionized water over a 53 μm sieve and thoroughly picked for roots. Roots were separated into fine (< 2 mm), coarse (2-5 mm), and very coarse (> 5mm) diameter fractions and dried at 55 ^{o}C before weighing (Baer et al. 2010). Dried fine roots were composited by plot across all three depths and ground before analyzing for total C and N and δ^{13}C isotopic composition (see *Laboratory Analyses*).

To determine aboveground *M. vimineum* biomass in each plot during the peak of the growing season in August 2017, I clipped all stems at ground level within two 25 x 25 cm quadrats randomly placed within the plot boundary. The samples were pooled by plot, dried at 60 ^{o}C to a constant mass and weighed. I randomly selected 12 pairs of plots and used the pooled samples from these plots to determine total C and N, and δ^{13}C (see *Laboratory Analyses*).

I sampled the forest floor by collecting the entire organic soil horizon ($O_i + O_e + O_a$) in each of the two 25 x 25 cm areas used to sample *M. vimineum* aboveground biomass at each plot. Samples were pooled by plot, dried at 60 ^{o}C to a constant mass and weighed. To estimate fuel consumption, I subtracted the litter mass of the burned plots from their ambient unburned pairs. This also provided a qualitative measure of difference in fire intensity because pre-burn litter mass is a strong predictor of fire temperature and residence time in this system (Wagner and Fraterrigo 2015).

To characterize the overstory tree community, I identified the species and measured the diameter at breast height (DBH) of every adult tree (> 10 cm DBH) within 50 m of the plot center (Appendix B). I also measured photosynthetically active radiation (PAR/PDIFF) to evaluate differences in understory light availability (LI-191R Line Quantum Sensor, LI-COR, Inc., Lincoln, NE). PAR was measured for each plot by standing at the plot center and extending the light sensor to each of the four corners of the plot in June and July 2017. After doing this four times, I averaged together the four measurements and subtracted this value from the measurement of full light PAR to calculate the PDIFF for each plot. I averaged the PDIFF values calculated from June and July prior to data analysis.

Laboratory Analyses

Each soil sample was analyzed for pH (2:1 mL H_2O:g soil) using a bench-top pH meter (Accumet AB15, Fisher Scientific, Waltham, MA), and total C and N and $\delta^{13}C$ of each fraction of SOM using a Costech 4010 CHNSO Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) interfaced with an isotope ratio mass spectrometer (Thermo Fisher Delta V Advantage, Fisher Scientific). Analytical error was <10% for total C and N and ± 0.2‰ for $\delta^{13}C$.

To determine the combined effects of invasion and fire on soil organic carbon (SOC) pools of differing stability, I fractionated soils into two SOM pools using the size-based procedure described in Bradford et al. (2008). SOM was separated into particulate organic matter (POM; > 53 μm) and mineral-associated organic matter (MAOM; < 53 μm). The POM fraction is composed of younger, less processed plant-derived organic matter that turns over relatively quickly. The MAOM fraction is composed of older, more microbially processed

organic matter that turns over more slowly (Schlesinger and Lichter 2001, Bradford et al. 2008). To separate these fractions, I dispersed 10 g samples with sodium hexametaphosphate by shaking samples for at least 18 hours, and physically separated fractions by washing them through a 53 μm sieve. POM samples were oven-dried at 115°C. A 130 mL subsample of the MAOM was collected from the homogenized solution that passed through the sieve, and was oven-dried at 60°C (Cambardella and Elliott 1992). Fractionated samples were ground prior to elemental and isotopic analysis.

To determine the contribution of *M. vimineum* to each fractionated SOC pool, I measured the $\delta^{13}C$ values of invaded and uninvaded POM-C and MAOM-C pools, and the *M. vimineum* leaf tissue. Deriving the relative contributions to SOC from *M. vimineum* is possible because *M. vimineum* uses a C_4-photosynthetic pathway, which distinguishes itself from the surrounding native C_3 plant community in the ratio of ^{13}C to ^{12}C. The mean $\delta^{13}C$ value for native POM (mean ± SE: -28.01‰ ± 0.11; $n = 33$) and native MAOM (-26.11‰ ± 0.15; $n = 33$) was greater than 3‰ different than *M. vimineum* leaf tissue (-13.35‰ ± 0.26; $n = 18$), making it sufficiently different to use for deriving contributions from different sources (Staddon 2004). The contributions of *M. vimineum* to soil POM-C and MAOM-C pools were calculated according to the following equation adapted from Ineson et al. (1996):

$$C_{M.vimineum\ derived} = C_{pool} * \frac{(\delta^{13}C_{invaded} - \delta^{13}C_{uninvaded})}{(\delta^{13}C_{M.vimineum} - \delta^{13}C_{uninvaded})}$$

where C_{pool} is the size of the C pool (POM or MAOM), $\delta^{13}C_{invaded}$ is the $\delta^{13}C$ value of the C pool in plots where *M. vimineum* is present, $\delta^{13}C_{uninvaded}$ is the $\delta^{13}C$ value of the C pool in plots where *M. vimineum* is absent, and $\delta^{13}C_{M.\ vimineum}$ is the $\delta^{13}C$ value for *M. vimineum* leaf tissue.

I measured active microbial biomass as the pulse of CO_2 after the addition of a high quality substrate to fresh soil by following the procedure for substrate-induced respiration (SIR) described in Fierer and Schimel (2003). Samples were incubated for four hours, and headspace concentrations of CO_2 were read using infrared gas analysis (LI-7000 CO_2/H_2O gas analyzer, LI-COR, Inc., Lincoln, NE). I measured microbial biomass C and N using the procedure for simultaneous chloroform fumigation extractions (sCFE) described in Fierer and Schimel (2003). Briefly, I combined pairs of fresh soil samples with 0.5 M K_2SO_4, while treating only one sample from each pair with 0.5 mL EtOH-free chloroform. Dissolved organic C and N were determined from the extracts using a TOC-L and TNM-L analyzer (Shimadzu Corporation, Kyoto, Japan). No correction factor was applied to the reported values for sCFE and SIR.

I analyzed whole soils for four extracellular enzyme activities associated with the breakdown of SOM with varying chemical complexity. β-glucosidase (BG) and β-N-acetylglucosaminidase (NAG) are hydrolytic enzymes that play a role in the turnover of faster-cycling SOM pools. BG primarily functions to hydrolyze cellulose into glucose (Ljungdahl and Eriksson 1985), while NAG is associated with N acquisition and the breakdown of chitin (Sinsabaugh et al. 2008). Phenol oxidase (PPO) and peroxidase (PER) are ligninolytic enzymes involved in the degradation of more complex and recalcitrant slow-cycling SOM pools (Weintraub et al. 2007). I followed the procedure from Finzi et al. (2006) for all enzyme assays. After a brief incubation, enzyme activities were calculated as the amount of substrate cleaved per unit mass of soil during the incubation period. BG and NAG were measured fluorimetrically at 365 nm excitation and 460 nm emission, and PPO and PER were measured spectrophotometrically at 460 nm emission using a microplate reader (BioTek Synergy HT Multi-Mode Microplate Reader, BioTek Instruments, Inc., Winooski, VT).

Data Analysis

I interpolated hourly soil temperature and moisture for each plot using the continuous datasets of soil temperature and moisture from each site to estimate CO_2 efflux for each plot at the hourly time scale. First, I filled any gaps in the continuous site-level data series by using simple linear regression against datasets from the Illinois State Water Survey (ISWS) stations (Water and Atmospheric Resources Monitoring Program, Illinois Climate Network, Illinois State Water Survey, Champaign, IL). The ISWS provided me with two continuous datasets from climate monitoring sites at DSSP and Carbondale, IL (10 miles north of GCSP), each consisting of hourly soil temperature under sod (10 cm depth) and soil moisture (avg. between 5 and 10 cm depth). Point measurements of CO_2 efflux, soil moisture, and soil temperature were averaged by plot before analysis. Next, I matched the point measurements to the corresponding date and hour within the continuous site-level dataset. I used simple linear regression between the point measurements and the continuous datasets to derive an hourly series of soil moisture and temperature values for each of the 40 plots. These continuous datasets of soil temperature and moisture were used to estimate the hourly soil CO_2 fluxes at each plot using the following natural log-linear quadratic model adapted from Martin and Bolstad (2005):

$$\ln(R_s) = b_0 + b_1(SWC) + b_2(SWC^2) + b_3(soilT) + b_4(soilT^2) + b_5(soilT * SWC)$$

where R_s is soil respiration, *SWC* is volumetric soil water content, and *soilT* is soil temperature. I used this equation because of the unimodal response of soil respiration to variation in moisture and temperature, while the natural logarithmic transformation of respiration corrects for any violation of homogeneity of variance (Bowden et al. 1998). Beta coefficients were derived for

each plot using point measurements of soil respiration, soil temperature, and soil moisture, and were used to predict a series of hourly soil respiration at each plot from June-November 2017 (Appendix C). Fluxes were converted from μmol CO_2 m^{-2} s^{-1} to g C m^{-2} hr^{-1}, and summed to determine cumulative flux per month.

I evaluated the effects of fire frequency and burn treatment on *M. vimineum* biomass and chemistry, cumulative soil CO_2 efflux, and microbial activities and soil chemistry using linear mixed effects models. I included treatment (ambient or burned), site-level fire frequency (frequent or excluded), soil depth (0-5 cm, 5-10 cm, 10-15 cm), and all possible interactions as fixed effects, specifying plot pair as a random intercept to account for potential spatial non-independence of the residuals. The spatial variation of environmental variables including soil temperature and light availability was greater between plot pairs within sites than between each site, which supports the assumption of independent sampling. Response variables were log transformed to meet the assumptions of normality and homogeneity of variance as necessary. To evaluate the effects of burn treatment at each level of fire frequency or depth increment, I performed planned contrasts while applying Tukey's HSD correction for multiple comparisons.

I used linear mixed effects models to assess whether invasion severity and soil moisture modulated the effects of fire frequency and burn treatment on enzyme activities. *M. vimineum* biomass, litter biomass, average SWC, and site-level fire frequency were included as main effects, and plot pair was included as a random effect. If any significant interactions were indicated, a post hoc analysis was used to compare the slopes of the fixed effects at each level of fire frequency. All variables were standardized and scaled prior to analysis to meet assumptions of normality and homoscedasticity and to enable direct comparison of effects sizes. All analyses

were performed in R (R Core Development Team, 2018) using the "lme4" and "lsmeans" packages.

Results

Microclimate and Soil Respiration

Soils were consistently drier in the plots that experienced frequent fire (DSSP) compared to the plots where fire was excluded prior to treatment in spring 2017 (GCSP; Table 2.1). Mean growing season soil volumetric water content (VWC) was 24.7 ± 0.75% (SE) at DSSP and 28.3 ± 1.1% at GCSP ($F_{1,18} = 7.07$, $p = 0.02$). Treatment effects on soil moisture varied with fire frequency ($F_{1,18} = 6.39$, $p = 0.02$). Under frequent fire, burning reduced mean soil VWC by 2.1 ± 0.9% v/v ($p = 0.04$) compared to ambient conditions. Burn treatment had no effect on soil moisture in the fire excluded plots. Neither mean growing season soil temperature nor PDIFF varied with fire frequency or burn treatment (Table 2.1).

Soil respiration (June-November 2017) increased with soil temperature ($R^2 = 0.37$) and maximum rates were measured in July at both sites (Fig. 2.1A). Cumulative respiration over the sampling period did not change in response to burn treatment in the plots that experienced frequent fire. However, burning reduced cumulative soil respiration by 24% compared to ambient plots in the fire excluded plots ($F_{1,18} = 13.05$, $p < 0.01$; Fig. 2.1B).

M. vimineum Root and Shoot Biomass

Aboveground biomass of *M. vimineum* was 87% higher in the fire excluded plots compared to the plots experiencing frequent fire ($F_{1,18} = 6.88$, $p = 0.02$; Fig. 2.2A). Mean aboveground biomass density of *M. vimineum* was 224.6 ± 33.4 (SE) g/m^2 at the fire excluded

plots compared to 119.9 ± 21.8 g/m^2 at the frequent fire plots. By contrast, fine root biomass was 85% higher in the frequent fire plots compared to the fire excluded plots ($F_{1,18} = 6.12$, $p = 0.02$; Fig. 2.2B). As a result, the root:shoot ratio for fine roots to a depth of 15 cm was almost 2.5 times higher in plots subjected to frequent fire compared to fire excluded plots ($F_{1,18} = 9.12$, $p < 0.01$). Burn treatment had no effect on aboveground or belowground biomass.

M. vimineum tissue stoichiometry also varied with fire frequency (Fig. 2.3A). Leaf C:N ratio was significantly lower in the fire excluded plots (15.6 ± 0.85 SE) compared to the frequent fire plots (18.5 ± 0.72; $F_{1,10} = 6.58$, $p = 0.03$). The C:N ratio of fine root biomass was also lower in the fire excluded plots (35.2 ± 2.4) than frequent fire plots (43.0 ± 1.6; $F_{1,18} = 7.13$, $p = 0.02$). Although burn treatment had no significant effect on leaf C:N ratio, it increased the C:N ratio of fine roots from 34.3 to 36.1 ± 2.8 in the fire excluded plots and from 40.0 to 45.9 ± 1.8 in the plots subjected to frequent fire compared to ambient plots (Fig. 2.3B; $F_{1,18} = 5.93$, $p = 0.03$).

Litter Biomass and Fuel Consumption

The fire excluded plots at GCSP had higher fine fuel loads than the frequent fire plots at DSSP as indicated by 35% higher litter biomass in ambient unburned plots at GCSP compared to DSSP (Table 2.1; $p = 0.05$). Although burning decreased litter biomass at both sites ($F_{1,18} = 21.23$, $p < 0.01$), litter consumption was nearly twofold greater in fire excluded plots (40.4 ± 16.1% SE) compared to the plots that experienced frequent fire (19.3 ± 10.5%); however, this difference was not statistically significant ($p = 0.29$).

Mineral Soil C and N Stocks

Burn treatment effects on soil pH depended on fire frequency ($F_{1,85} = 4.48$, $p = 0.04$). Compared to ambient conditions, burning increased soil pH from 5.50 to 5.66 ± 0.13 (SE) in the frequent fire plots ($p < 0.01$) and had no effect in the fire excluded plots, where soil pH averaged 5.71 ± 0.22 across treatments (Table 2.2).

Burn treatment had opposing effects on POM-C and POM-N stocks that varied with fire frequency (Table 2.2; $F_{1,85} = 7.48$, $p < 0.01$ and $F_{1,90} = 11.2$, $p < 0.01$, resp.). In the high fire frequency plots, burning increased POM-C by 16% ($p = 0.04$) and POM-N by 24% ($p < 0.01$) compared to the ambient treatment. In the fire excluded plots, however, burning decreased POM-C by 20% ($p = 0.06$) and POM-N by 18% ($p = 0.06$) compared to the ambient treatment. As a result, the C:N ratio of the POM fraction post-burning was similar across plots, decreasing on average from 22.4 to 20.7 in burned plots compared to ambient plots (Table 2.2; $F_{1,85} = 6.82$, $p = 0.01$).

Burn treatment also had contrasting effects on MAOM-C and MAOM-N stocks that varied with fire frequency (Table 2.2; $F_{1,85} = 6.15$, $p = 0.02$ and $F_{1,90} = 5.61$, $p = 0.03$, resp.). In the plots subjected to frequent fire, burning increased MAOM-C by 10% ($p < 0.01$) and MAOM-N by 7% ($p = 0.02$) compared to the ambient treatment; burning had no significant effects in the fire excluded plots (Table 2.2). As a result, the C:N ratio of the MAOM fraction increased significantly in burned compared to ambient plots in the frequent fire plots ($p = 0.03$). Across treatments, the C:N ratio of the MAOM fraction was marginally higher at in the plots that experienced frequent fire (9.81 ± 0.17) compared to the fire excluded plots (9.24 ± 0.26; $F_{1,17} = 3.28$, $p = 0.09$).

DOC concentration was 55% higher on average in the plots experiencing frequent fire compared to the fire excluded plots (Table 2.2; $F_{1,16} = 11.63$, $p < 0.01$). In contrast, DON concentration was 22% higher on average in the surface mineral soil (0-5 cm) in the fire excluded plots compared to frequent fire plots ($p = 0.05$). Burn treatment had no significant effect on DOC or DON at either site.

Burning marginally decreased the percentage of *M. vimineum*-derived C found in soil organic C pools regardless of fire frequency (Table 2.3). Compared to the ambient treatment, there was 19% less *M. vimineum*-derived C in the POM-C pool ($F_{1,85} = 2.91$, $p = 0.09$) and 27% less in the MAOM-C pool ($F_{1,85} = 3.22$, $p = 0.08$). There was a non-significant trend toward increasing *M. vimineum*-derived C in the surface POM-C ($\beta = 0.29$, $p = 0.06$) and MAOM-C ($\beta = 0.30$, $p = 0.09$) pools with increasing aboveground *M. vimineum* biomass, irrespective of burn treatment and fire frequency.

Soil Microbial Activity

Active microbial biomass, as measured by SIR, was 96% higher on average in the plots subjected to frequent fire compared to the fire excluded plots but this trend was not statistically significant (Table 2.4; $F_{1,18} = 1.57$, $p = 0.10$). The effects of burn treatment varied with fire frequency ($F_{1,90} = 10.3$, $p < 0.01$). Burning increased active microbial biomass in the frequent fire plots by 39% compared to ambient plots ($p < 0.01$), and had no significant effect in the fire excluded plots. Microbial biomass C:N was significantly higher in the frequent fire plots (5.54 ± 0.35 SE) compared to the fire excluded plots (4.09 ± 0.40; $F_{1,18} = 6.83$, $p = 0.02$), and burning marginally increased microbial C:N by 15% ($F_{1,90} = 3.49$, $p = 0.07$) compared to ambient plots across both sites (Table 2.4).

The activities of hydrolytic enzymes were generally higher in the plots subjected to frequent fire compared to the fire excluded plots (Table 2.4). Burning enhanced these differences, increasing BG activity by 34% compared to ambient plots in the frequent fire plots ($p < 0.01$), while having no effect in the fire excluded plots. NAG activity was 59% higher in the frequent fire plots than the fire excluded plots ($F_{1,18} = 4.83$, $p = 0.04$). Specific activities of BG and NAG did not differ between treatments or by fire frequency. *M. vimineum* biomass was negatively related to BG ($\beta = -0.462$, $p = 0.01$) and NAG ($\beta = -0.519$, $p < 0.01$) activities across both sites in the 0-5 cm soil layer (Fig. 2.5).

Oxidase enzyme activities varied with fire frequency in the deepest soil layer (10-15 cm), with PPO ($F_{2,65} = 4.21$, $p = 0.02$) and PER ($F_{2,75} = 3.37$, $p = 0.04$) activities elevated in the fire excluded plots compared to the plots subjected to frequent fire (Table 2.4). Specific PER activity was also 54% higher in the fire excluded plots averaged across soil depths ($F_{1,15} = 35.44$, $p = 0.04$).

Discussion

Positive resource-based feedbacks can sustain the long-term persistence of invasive plants and help maintain their dominance over native plant communities (Morris et al. 2016, Zhang et al. 2019). They can also lead to changes in biogeochemical cycling that alter ecosystem functioning. Yet few studies have investigated how resource-based feedbacks and their potential impacts on ecosystem functioning change over time in the presence of increasing fire activity. Here, I found that *M. vimineum*-invaded plots experiencing frequent fire had populations with lower biomass production and higher root:shoot and tissue C:N ratios. I also found higher microbial biomass C:N ratios and elevated activities of the N-degrading enzyme

NAG in plots subjected to frequent fire. Additionally, the effect of experimental burning often depended on site-level fire frequency, with burning resulting in increased soil C and N stocks in plots with a history of frequent fire but neutral or opposing effects in fire exclusion plots. Together, these findings support my hypothesis that frequent fire weakens resource-based plant-soil feedbacks and diminishes the biogeochemical effects of invasion by exacerbating N deficiency.

The observed patterns in *M. vimineum* allometry and tissue stoichiometry suggest that population vigor was declining in response to increasing N deficiency. Studies in temperate deciduous forests consistently show a negative relationship between fine root biomass and soil N availability (Walters and Reich 1997, Coomes and Grubb 2000), while plant foliar C:N ratio is shown to increase under greater N stress (Greenwood 1976, Sterner and Elser 2002). Similar patterns in growth are shown to occur with other species of non-native plants, where long-term declines in populations are observed in association with a weakening of resource-based plant-soil feedbacks caused by depletion of soil N (Yelenik and D'Antonio 2013).

Consistent with the hypothesis that frequent fire increases N deficiency, I found elevated NAG enzyme activity, lower DON in the surface soil, and higher microbial biomass C:N within the mineral soil of the frequent fire plots compared to the fire exclusion plots. Elevated NAG activity indicates that soil microbial decomposers are producing extracellular enzymes to mobilize and acquire more N, as suggested by studies that observe NAG activity to be significantly negatively correlated with soil N availability (Brockett et al. 2012, Rietl and Jackson 2012). The hypothesis that repeated burning may progressively deplete soil N availability by volatilizing N stored in biomass has also been tested in cheatgrass invaded systems, but fire temperatures ultimately fell below the N volatilization temperatures of 200^{o}C

and did not result in progressive losses of N (Jones et al. 2015). Although I did not measure fire temperatures during in this study, previous research at DSSP has shown maximum soil surface temperatures to reach upwards of 300^{o}C (Wagner and Fraterrigo 2015), well above the temperature required to induce N losses through volatilization (Raison et al. 1985).

The observed patterns in *M. vimineum* allometry and tissue stoichiometry also align with theoretical predictions of shifting microbial CUE and NUE in response to altered substrate C:N ratios. Under the stoichiometric theory of ecology, microbial growth becomes N limited when the availability of N relative to C falls below the threshold elemental ratio (TER). This critical C:N ratio is empirically determined to be around 20-25 for terrestrial systems, although the TER is highly variable depending on decomposer biomass C:N and initial substrate stoichiometry (Sinsabaugh et al. 2013). When resource C:N conditions are above the TER, N is primarily immobilized as microbes express maximum NUE under N limitation (Mooshammer et al. 2014a). Repeated fire is likely to alter resource conditions conducive to increases in microbial NUE, and previous studies have shown repeated fire to increase plant C:N ratios resulting in lower N mineralization belowground (Ojima et al. 1994). Therefore, this change in microbial resource use efficiency may contribute to greater weakening of resource-based plant-soil feedbacks as N is depleted during repeated burning.

Patterns observed in microbial properties and soil C cycling reinforce the hypothesis that microbial resource use efficiencies differ depending on fire frequency. Elevated active microbial biomass at the site with frequent fire provides an indirect measurement of microbial CUE, and shows a heightened release of CO_2 in the presence of high-quality substrate, consistent with microbes expressing a low CUE and high NUE under conditions of greater N limitation. This is also supported by the trends I observed in soil respiration, where burning resulted in a significant

reduction in soil CO_2 efflux at the fire excluded site. This signal can be attributed primarily to a response in heterotrophic respiration since I did not find any differences in root biomass between burn treatments. A reduction in soil CO_2 efflux with burning at fire excluded plots suggests a greater effect of fire at the site where microbial communities are less N limited and less likely to be comprised of fire-tolerant species. In response to a pulse of mineralized N mobilized by fire, microbial communities may be responding by downregulating NUE and upregulating CUE, leading to decreases in C lost through respiration. Meanwhile, microbial communities at the high fire frequency site are more fire tolerant and more N limited, while already expressing a maximum NUE, evidenced by a lack of change in soil respiration.

Because of limited microbial stoichiometric flexibility, fire may also shift microbial communities from being bacterial- to fungal-dominated, as fungal communities tend to have higher C:N ratios compared to bacterial communities (Sterner and Elser 2002). Supporting this, I found higher microbial biomass C:N ratios in frequent fire plots compared to fire excluded plots, and I observed that burning marginally increased microbial biomass C:N ratios at each site, suggesting that even after a single burn, microbial communities may be shifting toward more fire-tolerant functional communities with a higher biomass C:N ratio. Long-term studies have shown these types of microbial community shifts to persist with repeated burning (Oliver et al. 2015), while single burns may have positive short-term effects on fungal species richness as well (Smith et al. 2004). Increased fungal dominance is also consistent with elevated NAG activity at the frequent fire plots, which is an indicator of soil fungal abundance (Miller et al. 1998).

These results also show that *M. vimineum* biomass varied negatively with both BG and NAG enzyme activities, while soil moisture remained an insignificant predictor for both enzymes. Based on previous studies focusing on *M. vimineum*, enzyme activities tend to be

higher under *M. vimineum* invasion when N is limiting, supporting the hypothesis that invasion drives increases in microbial activity and turnover of SOM via plant-microbial competition for N (Craig and Fraterrigo 2017). However, I observed a negative relationship between *M. vimineum* and BG and NAG activity, revealing that where *M. vimineum* production is high, hydrolytic enzyme activities are suppressed. High growth of *M. vimineum* is likely an indication of greater N availability, which may result in downregulation of enzymes produced to mobilize N. This is also important to consider within the context of repeated fire effects. If fire is diminishing N availability and causing an increase in microbial NUE, there may be cascading effects on microbial activity and storage of soil C and N in the long-term. However, additional research is needed to investigate this hypothesis.

Shifts in soil microbial resource use efficiencies associated with altered resource conditions may explain the observed differences in biogeochemical cycling with fire frequency and experimental burning. Burning in the invaded plots with a history of frequent fire significantly increased soil C and N stocks in both the POM and MAOM fractions. By contrast, burning led to different effects in the invaded, fire excluded plots: soil CO_2 efflux and POM C and N stocks decreased in response to fire. These results suggest that fire history mediates the ecosystem response to burning through the fire legacy effects of diminished N availability and increased microbial NUE (Cheng et al. 2013). Alternatively, fire excluded plots may have experienced higher fire intensity during burning resulting from greater surface litter accumulation and *M. vimineum* productivity, leading to further contrasting effects of fire at each site. The increase in SOM in the plots with frequent fire may have resulted from deposition of partially combusted organic materials and increased chemical complexity of existing SOM that can occur with low-intensity fire, which slows the rate of degradation (Gonzalez-Perez et al.

2004, Marschner et al. 2008). In contrast, burning had a negative effect on POM-C and N stocks at plots with fire exclusion, where higher fire intensity most likely increased combustion and volatilization of POM-C and N (Fernandez et al. 1997, Certini 2005). Despite opposing changes in POM-C and N stocks depending on fire frequency, unique volatilization temperatures for C and N are likely the biggest drivers of post-fire decrease in POM-C:N observed across both frequent fire and fire excluded plots (Butler et al. 2017). Although I did not detect differences in stocks organic C and N between frequent fire and fire excluded plots, future studies should investigate whether changes in CUE and NUE associated with long term repeated burning and invasion ultimately have consequences for SOM storage.

Synthesis and Conclusion

These findings provide insight into how invasive plant-soil feedbacks can be altered through disturbance-mediated feedbacks like fire, and highlights the importance of determining how plant invasions decline or persist over extended periods and disturbances. The results from this study support the hypothesis that repeated fire may increase losses of ecosystem N made vulnerable to loss through invasion by a non-native grass, and suggests that frequent fire could mitigate invasion impacts by weakening resource-based plant-soil feedbacks that alter soil biogeochemical cycles to promote invader growth. Increasingly, long-term studies are finding that self-reinforcing feedbacks of invasions are dynamic through time (Yelenik and D'Antonio 2013, Flory et al. 2017), and this study improves our understanding of how fire can be a mechanism for driving these changes in invasive plant persistence.

Figures and Tables

Table 2.1 Summary of average soil water content, soil temperature, PDIFF, and litter biomass (mean ± 1 SE), with significant main effects or interactions from the linear mixed effects model indicated by asterisks († $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Site:	**Frequent Fire (DSSP)**		**Fire Excluded (GCSP)**		Significant Effects:		
Treatment:	**Ambient**	**Burned**	**Ambient**	**Burned**	*Site*	*Treatment*	*Site x Treatment*
7.5 cm Soil Water Content (m^3 m^{-3})	0.257 ± 0.009	0.236 ± 0.008	0.270 ± 0.014	0.293 ± 0.015	*		*
10 cm Soil Temperature (^oC)	20.5 ± 0.1	20.9 ± 0.1	20.3 ± 0.2	20.4 ± 0.2			
PDIFF (Full Light - PAR; µmol s^{-1} m^{-2})	1330 ± 120	1270 ± 120	1140 ± 190	960 ± 190			
Litter Biomass (g m^{-2})	352 ± 33	257 ± 33	475 ± 51	270 ± 51		***	

Figure 2.1 Modeled cumulative soil respiration by month (g C m^{-2} $month^{-1}$; mean ± 1 SE) (A), and by the entire study period from June-November 2017 (g C m^{-2}; mean ± 1 SE) (B). Results are separated by site (DSSP or GCSP) and burn treatment. Cumulative respiration was modeled using a log-linear quadratic model based on hourly soil temperature and soil moisture data from June-November 2017 († $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

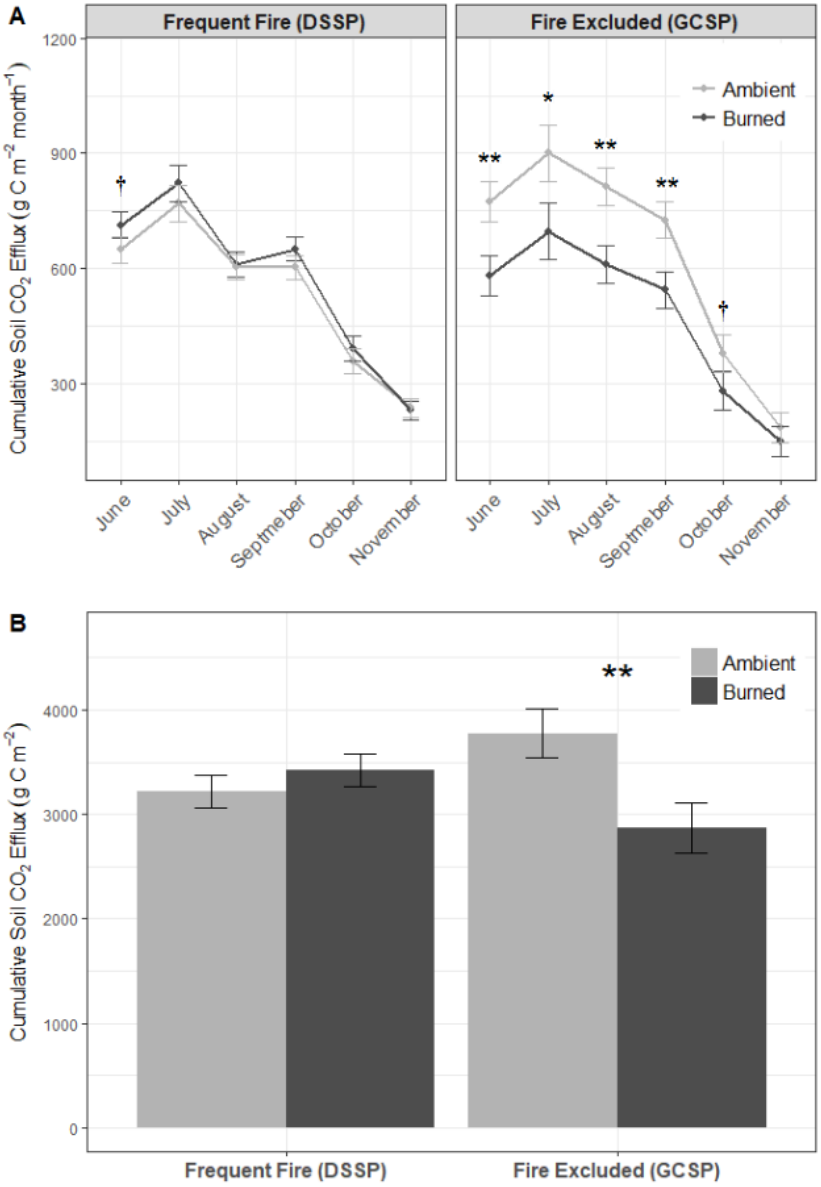


Figure 2.2 Aboveground *M. vimineum* biomass (g m^{-2}; mean ± 1 SE) (A) and fine root (< 2 mm) biomass (B) at invaded study sites with frequent fire (DSSP) or fire excluded (GCSP) in August 2017. Burned plots were treated with a prescribed burn in April 2017. Values are presented as dry weight biomass after oven dried to constant mass († $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

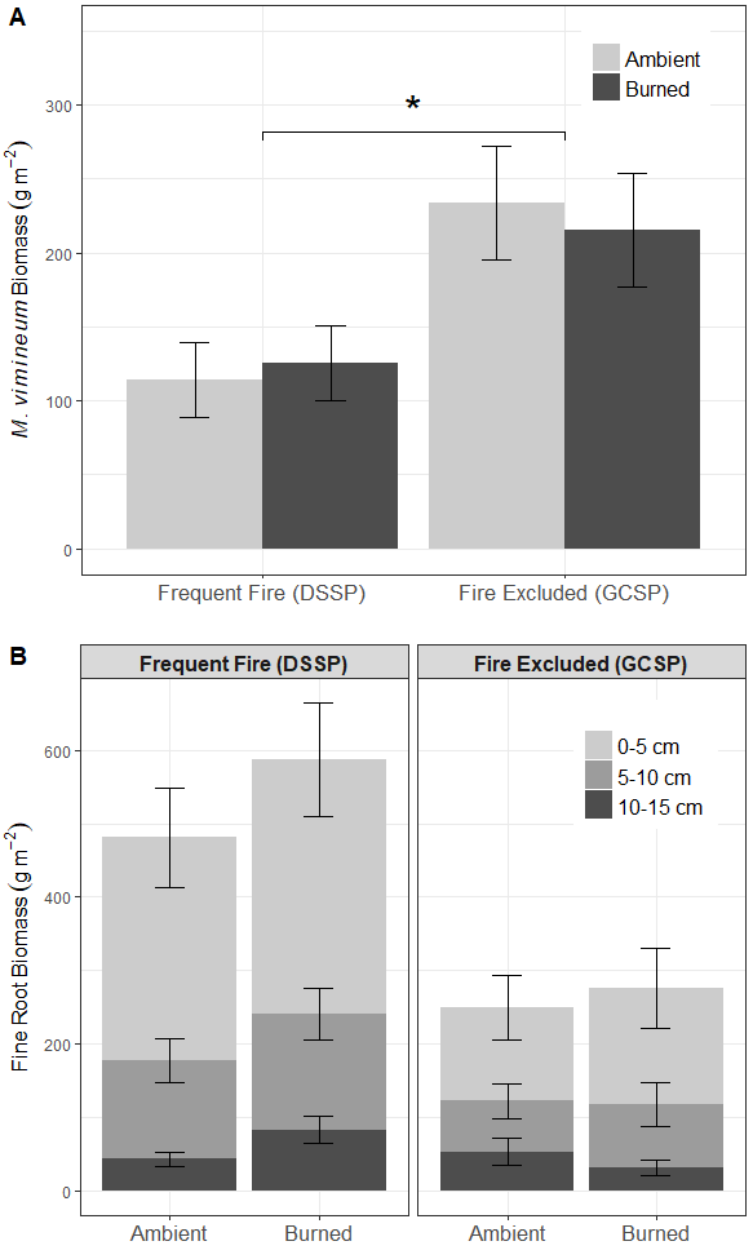


Figure 2.3 *M. vimineum* foliage C:N (g m^{-2}; mean ± 1 SE) (A) and fine root (< 2 mm) C:N (B) at invaded study sites with frequent fire (DSSP) or fire excluded (GCSP) in August 2017. Burned plots were treated with a prescribed burn in April 2017. Values are presented as dry weight biomass after oven dried to constant mass († $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

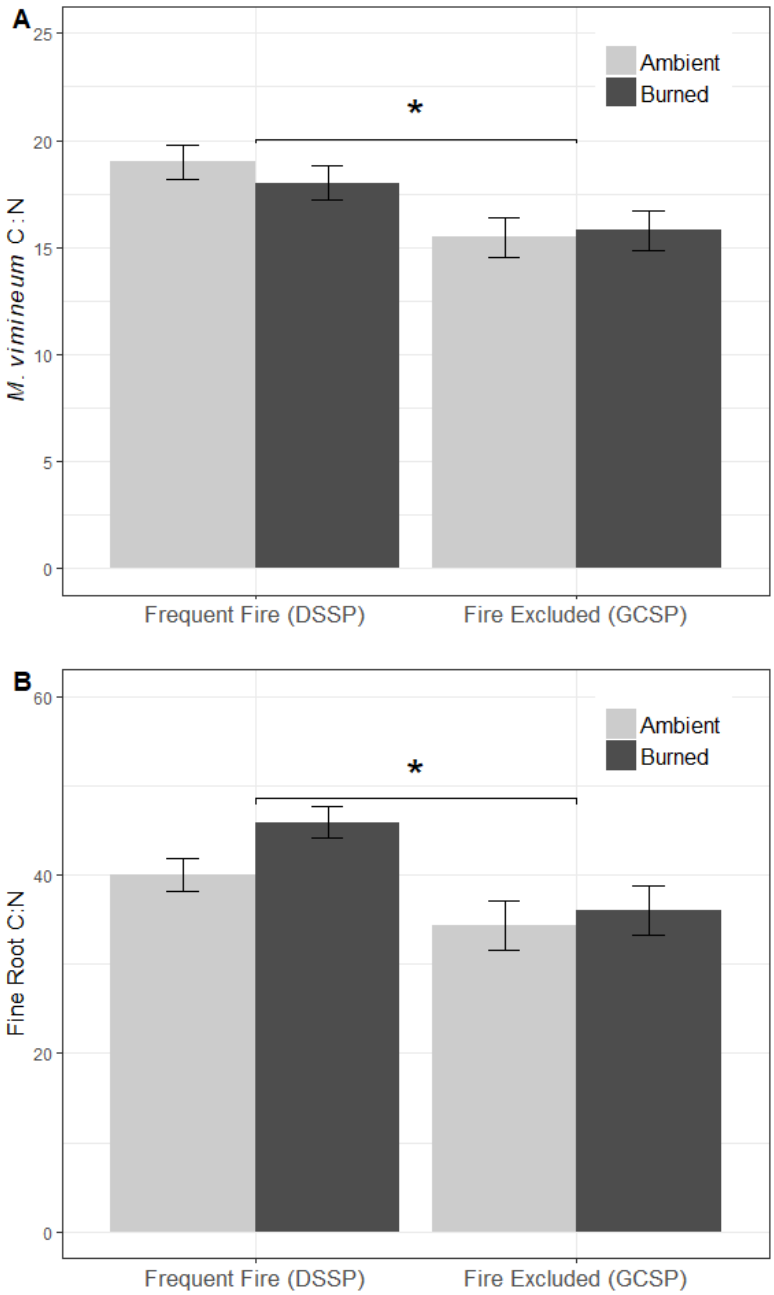


Table 2.2 Summary of soil pH, mineral soil C and N stocks, DOC and DON concentrations (mean ± 1 SE) with significant main effects or interactions from the linear mixed effects model indicated by asterisks († $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Site:		Frequent Fire (DSSP)		Fire Excluded (GCSP)		Significant Effects:					
Treatment:		Ambient	Burned	Ambient	Burned	*Site*	*Treatment*	*Depth*	*Site x Treatment*	*Site x Depth*	*Site x Treatment x Depth*
Soil pH	0-5 cm	5.66 ± 0.15	5.76 ± 0.15	5.87 ± 0.25	5.70 ± 0.25			*	*		
	5-10 cm	5.50 ± 0.14	5.68 ± 0.15	5.71 ± 0.25	5.68 ± 0.24						
	10-15 cm	5.36 ± 0.14	5.55 ± 0.14	5.68 ± 0.24	5.64 ± 0.24						
POM-C ($g\ C\ m^{-2}$)	0-5 cm	656.94 ± 61.32	682.80 ± 63.74	587.68 ± 91.80	574.15 ± 89.68			***	**		†
	5-10 cm	174.44 ± 16.28	207.70 ± 19.39	202.63 ± 31.65	106.38 ± 16.62						
	10-15 cm	86.46 ± 8.07	108.57 ± 10.13	88.36 ± 13.80	87.39 ± 13.65						
POM-N ($g\ N\ m^{-2}$)	0-5 cm	33.67 ± 3.14	36.96 ± 3.45	29.22 ± 4.17	29.16 ± 4.16			***	**		
	5-10 cm	8.29 ± 0.77	10.76 ± 1.00	9.41 ± 1.34	6.14 ± 0.87						
	10-15 cm	3.31 ± 0.31	4.47 ± 0.42	4.06 ± 0.58	3.40 ± 0.48						
POM C:N (*atomic ratio*)	0-5 cm	19.51 ± 0.89	18.47 ± 0.84	20.89 ± 1.59	19.56 ± 1.49		*	***			
	5-10 cm	21.05 ± 0.96	19.30 ± 0.88	22.65 ± 1.73	18.88 ± 1.44						
	10-15 cm	26.12 ± 1.19	24.30 ± 1.11	24.81 ± 1.89	24.88 ± 1.90						
MAOM-C ($g\ C\ m^{-2}$)	0-5 cm	1133.76 ± 60.13	1183.91 ± 62.78	994.87 ± 88.28	989.55 ± 87.81			***	*		
	5-10 cm	749.72 ± 39.76	833.91 ± 44.22	763.34 ± 67.74	667.43 ± 59.23						
	10-15 cm	522.42 ± 27.70	595.25 ± 31.57	517.10 ± 45.89	498.19 ± 44.21						
MAOM-N ($g\ N\ m^{-2}$)	0-5 cm	108.5 ± 5.07	111.82 ± 5.22	103.73 ± 7.40	102.62 ± 7.32			***	*		
	5-10 cm	78.54 ± 3.67	85.48 ± 3.99	86.14 ± 6.15	78.72 ± 5.62						
	10-15 cm	57.20 ± 2.67	62.75 ± 2.93	60.97 ± 4.35	57.66 ± 4.11						
MAOM C:N (*atomic ratio*)	0-5 cm	10.45 ± 0.22	10.59 ± 0.22	9.98 ± 0.35	9.91 ± 0.35	†		***	*		
	5-10 cm	9.55 ± 0.20	9.76 ± 0.20	9.32 ± 0.33	8.73 ± 0.30						
	10-15 cm	9.13 ± 0.19	9.49 ± 0.20	8.79 ± 0.31	8.79 ± 0.31						
DOC ($\mu g\ C\ g\ soil^{-1}$)	0-5 cm	106.72 ± 11.74	107.75 ± 11.85	84.29 ± 17.35	67.94 ± 13.98	**		***			
	5-10 cm	73.14 ± 8.05	90.50 ± 9.96	49.15 ± 10.12	45.49 ± 9.36						
	10-15 cm	77.93 ± 8.57	80.34 ± 8.84	42.73 ± 8.79	40.53 ± 8.34						
DON ($\mu g\ C\ g\ soil^{-1}$)	0-5 cm	23.23 ± 2.68	23.59 ± 2.73	30.25 ± 5.14	25.12 ± 4.27			***	*	†	
	5-10 cm	13.66 ± 1.58	14.89 ± 1.72	14.23 ± 2.42	8.70 ± 1.48						
	10-15 cm	8.70 ± 1.00	9.93 ± 1.15	9.07 ± 1.54	8.69 ± 1.48						

Table 2.3 Summary of *M. vimineum*-derived POM-C and MAOM-C (mean ± 1 SE), with significant main effects or interactions from the linear mixed effects model indicated by asterisks († $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Site:		Frequent Fire (DSSP)		Fire Excluded (GCSP)		Significant Effects:					
Treatment:		**Ambient**	**Burned**	**Ambient**	**Burned**	*Site*	*Treatment*	*Depth*	*Site x Treatment*	*Site x Depth*	*Site x Treatment x Depth*
M. vimineum-derived POM-C (%)	0-5 cm	11.6 ± 1.9	8.5 ± 1.9	13.0 ± 3.2	12.4 ± 3.2		†	***			
	5-10 cm	4.7 ± 1.9	3.8 ± 1.9	4.8 ± 3.2	5.8 ± 3.2						
	10-15 cm	3.5 ± 1.9	2.0 ± 1.9	7.2 ± 3.2	3.7 ± 3.2						
M. vimineum-derived MAOM-C (%)	0-5 cm	6.5 ± 1.3	5.6 ± 1.3	5.8 ± 2.2	5.5 ± 2.2		†	**			
	5-10 cm	4.3 ± 1.3	1.8 ± 1.3	4.2 ± 2.2	5.4 ± 2.2						
	10-15 cm	4.5 ± 1.3	1.6 ± 1.3	4.3 ± 2.2	1.8 ± 2.2						

Figure 2.4 Fire-induced change (burned – ambient) in POM-C:N (A), MAOM-C:N (B), and microbial biomass C:N (C). Values are presented as atomic ratio means (± 1 SE), where values above zero indicate a shift to higher C:N ratios with fire, and values below zero indicate a shift toward lower C:N ratios with fire.

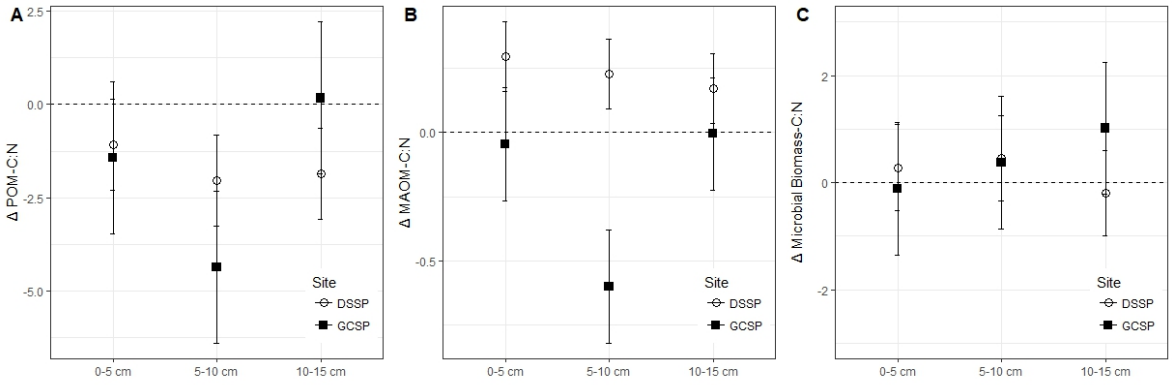
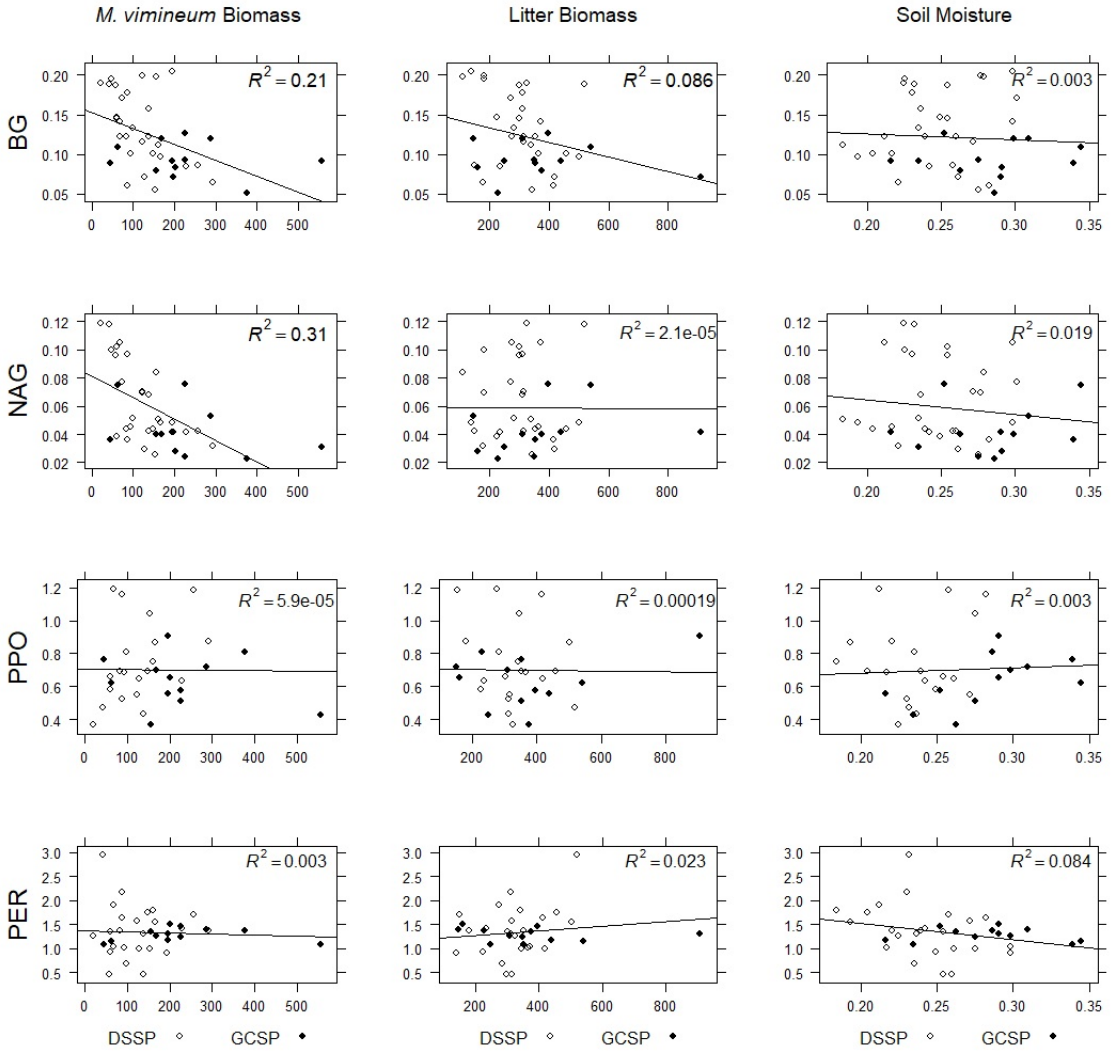


Table 2.4 Summary of microbial biomass C and N concentrations measured by sCFE, active microbial biomass measured by SIR, and extracellular enzyme activities (mean ± 1 SE), with significant main effects or interactions from the linear mixed effects model indicated by asterisks († $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Site:		Frequent Fire (DSSP)		Fire Excluded (GCSP)		Significant Effects:				
Treatment:		Ambient	Burned	Ambient	Burned	*Site*	*Treatment*	*Depth*	*Site x Treatment*	*Site x Depth*	*Site x Treatment x Depth*
Microbial Biomass-C ($\mu g\ C\ g\ soil^{-1}$)	0-5 cm	161.02 ± 26.99	186.80 ± 31.31	146.82 ± 36.22	131.90 ± 32.54	†		***			
	5-10 cm	66.23 ± 11.10	87.12 ± 14.60	68.11 ± 16.80	54.57 ± 13.46						
	10-15 cm	37.74 ± 6.33	41.30 ± 6.92	21.10 ± 5.21	33.35 ± 8.23						
Microbial Biomass-N ($\mu g\ N\ g\ soil^{-1}$)	0-5 cm	32.80 ± 3.96	37.03 ± 4.47	39.68 ± 7.06	32.55 ± 5.79			***	†		
	5-10 cm	11.71 ± 1.41	13.79 ± 1.67	14.48 ± 2.57	12.11 ± 2.15						
	10-15 cm	6.45 ± 0.78	7.49 ± 0.90	7.09 ± 1.26	6.77 ± 1.20						
Microbial Biomass C:N (atomic ratio)	0-5 cm	4.76 ± 0.53	5.07 ± 0.56	3.70 ± 0.63	4.05 ± 0.69	*	†				
	5-10 cm	5.53 ± 0.61	6.37 ± 0.70	4.70 ± 0.80	4.50 ± 0.76						
	10-15 cm	5.63 ± 0.62	6.07 ± 0.67	2.98 ± 0.50	4.93 ± 0.83						
Active Microbial Biomass ($\mu g\ CO_2\text{-}C\ g\ soil^{-1}\ hr^{-1}$)	0-5 cm	38.96 ± 9.20	56.14 ± 13.26	22.48 ± 8.11	20.3 ± 7.32	†		***	**		
	5-10 cm	12.27 ± 2.90	17.66 ± 4.17	8.57 ± 3.09	5.91 ± 2.13						
	10-15 cm	6.39 ± 1.51	8.32 ± 1.97	5.02 ± 1.81	3.86 ± 1.39						
β-Glucosidase (BG) Activity ($nmol\ g\ soil^{-1}\ hr^{-1}$)	0-5 cm	0.119 ± 0.015	0.131 ± 0.016	0.100 ± 0.019	0.083 ± 0.016			***	***	†	
	5-10 cm	0.034 ± 0.004	0.051 ± 0.006	0.039 ± 0.007	0.030- ± 0.006						
	10-15 cm	0.017 ± 0.002	0.025 ± 0.003	0.022 ± 0.004	0.021 ± 0.004						
β-N-Acetylglucosaminidase (NAG) Activity ($nmol\ g\ soil^{-1}\ hr^{-1}$)	0-5 cm	0.057 ± 0.008	0.061 ± 0.009	0.048 ± 0.010	0.032 ± 0.007	*		***	†		
	5-10 cm	0.025 ± 0.004	0.028 ± 0.004	0.017 ± 0.004	0.014 ± 0.003						
	10-15 cm	0.019 ± 0.003	0.020 ± 0.003	0.012 ± 0.003	0.013 ± 0.003						
Phenol Oxidase (PPO) Activity ($nmol\ g\ soil^{-1}\ hr^{-1}$)	0-5 cm	0.689 ± 0.101	0.719 ± 0.105	0.598 ± 0.124	0.607 ± 0.126	†				*	
	5-10 cm	0.580 ± 0.085	0.785 ± 0.115	0.873 ± 0.181	0.923 ± 0.191						
	10-15 cm	0.451 ± 0.066	0.534 ± 0.078	0.982 ± 0.203	0.719 ± 0.149						
Peroxidase (PER) Activity ($nmol\ g\ soil^{-1}\ hr^{-1}$)	0-5 cm	1.344 ± 0.148	1.157 ± 0.127	1.227 ± 0.209	1.279 ± 0.218	†				*	
	5-10 cm	0.944 ± 0.104	1.253 ± 0.138	1.365 ± 0.232	1.304 ± 0.222						
	10-15 cm	0.830 ± 0.091	0.947 ± 0.104	1.545 ± 0.263	1.327 ± 0.226						

Figure 2.5 Extracellular enzyme activities for β-glucosidase (BG), β-1,4-N-acetylglucosaminidase (NAG), phenol oxidase (PPO), and peroxidase (PER) (nmol substrate cleaved g^{-1} soil hr^{-1}) in the surface (0-5 cm) soil layer plotted by *M. vimineum* biomass (g m^{-2}), litter biomass (g m^{-2}), and average volumetric soil water content. R^2 values shown in bold represent significant effects ($p < 0.05$) from the linear mixed effects models for predicting enzyme activities.



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CHAPTER 3: DROUGHT ALTERS PLANT-SOIL FEEDBACKS OF A NON-NATIVE INVASIVE GRASS

Abstract

Non-native invasive plants can alter ecosystem processes by establishing self-reinforcing feedbacks with soil microbial communities. However, changes in abiotic conditions associated with frequent or prolonged drought may alter these biogeochemical feedbacks by diminishing microbial activities, resulting in changes in invasive plant productivity and the ecosystem impacts of invasion. Using rainout shelters, I imposed growing season drought on populations of the exotic grass *Microstegium vimineum* in forests with differing levels of soil nitrogen availability. I measured plant productivity, soil carbon (C) and nitrogen (N) pools, soil CO_2 efflux, soil microbial biomass, and extracellular enzyme activities across sites with differing N limitation status to evaluate the hypothesis that drought reduces invader productivity and ecosystem impacts by decreasing the activities of microbial decomposers. I found that moisture limitation contributes negatively to invasive plant-soil feedbacks and may be co-limiting with soil nitrogen (N) to constrain invader impacts and productivity. My results show that drought significantly reduced soil respiration and *M. vimineum* productivity, while increasing *M. vimineum* leaf C:N ratio at the site with lower N limitation. I also found that drought decreased C:N ratio of the soil particulate organic matter (POM) fraction and decreased concentrations of dissolved organic nitrogen (DON), but had no effect on total C and N stocks. These results suggest that although climate change is predicted to facilitate the spread of invasive plants in the future, increases in the frequency and duration of droughts may reduce the vigor of invasive species populations by weakening plant-soil feedbacks.

Introduction

Invasive plants and climate change represent pervasive threats to ecosystem structure and functioning and will continue to interact at an increasingly higher rate in the future (Walther et al. 2009, Hulme 2017). Invasive plants often maintain their persistence in novel ecosystems by shifting functional traits in a community to establish positive plant-soil feedbacks (Ricciardi et al. 2017, Zhang et al. 2019). Invasive plants can alter plant-soil feedbacks through higher growth rates and resource use efficiencies that inherently increase the quantity and quality of inputs to the soil relative to native species (Liao et al. 2008, Castro-Diez et al. 2014). This can initiate a positive resource-mediated feedback that may push ecosystems toward alternative stable states favoring the dominance of invasives over native species (Bever et al. 1997, Ehrenfeld et al. 2005, van der Putten et al. 2013). Although the existence of these invasive plant-soil feedbacks are well studied, it is not fully understood how climatic changes could mediate these biogeochemical feedbacks in the future.

Many aspects of climate change, including changing precipitation regimes, are expected to facilitate the spread of invasive plants in the future (Weltzin et al. 2003, Bradley et al. 2010, Sorte et al. 2013, Liu et al. 2017). Climate models specifically predict an increase in frequency and duration of droughts across regions in the U.S. resulting from decreased precipitation and increased evapotranspiration due to warming (Dai 2013, Trenberth et al. 2014), with a particular increase in drying over the central U.S. during the summer (Singh et al. 2013). These hydrological shifts are expected to have far-reaching consequences for ecosystem functioning and biogeochemical cycles (Reichstein et al. 2013, Anderegg et al. 2015, Frank et al. 2015), and may reduce plant productivity enough to shift ecosystems from carbon (C) sinks to C sources (Ciais et al. 2005). While plant invasion and drought are predicted to co-occur at an increasing

rate, the combined effects of these stressors on ecosystem functioning are unlikely to be simply additive (Alba et al. 2017).

Interactions between invasive plants and droughts could be synergistic or antagonistic when their combined effects on ecosystem functioning are greater or less than the predicted additive effects (Cote et al. 2016). Under synergistic conditions, the effects of non-native plants may be more prominent under drought conditions, where native species are less resistant to abiotic stress. Yet under antagonistic conditions, invasive plants may instead ameliorate drought stress by decreasing evaporation from the soil surface and increasing humidity below the canopy. Previous studies have shown grass invasion to ameliorate drought stress by maintaining higher soil moisture relative to uninvaded areas (Fahey et al. 2018). However, invaders may also interact synergistically with drought events to further suppress native species success while maintaining self-reinforcing feedbacks (Caldeira et al. 2015, Manea et al. 2016).

Within the soil, moisture levels represent one of the strongest controls on microbial community characteristics and nutrient cycling (Tiemann and Billings 2011, Brockett et al. 2012), where drought may leave a legacy of effects on the ecosystem up to several years after the event (Anderegg et al. 2015). Decreases in soil moisture tend to slow the rate at which soil processes occur and decrease the abundance of soil organisms (Kardol et al. 2010), which could limit the capacity of resource-mediated feedbacks driven by plant invasions by reducing the rate of decomposition and mineralization of nutrients. Soil moisture limitation may also alter individual plant function and physiology in ways that indirectly affect the soil microbial ecosystem by limiting supply of plant-derived C to the soil (Yuste et al. 2007). By limiting productivity of the plant community, this may alter the quantity and quality of detrital inputs to

the soil leading to decreased mineralization of soil C and nitrogen (N) (Cotrufo and Ineson 1995).

A growing body of literature suggests that primary productivity is likely to be co-limited by multiple resources at the same time, specifically N and water in temperate systems (Harpole et al. 2007, Fay et al. 2015, Lu et al. 2018). For example, total annual net primary productivity in temperate steppe is shown to be sub-additively co-limited by N and moisture availability, where ecosystems responded greater to water addition than N addition during dry years while responding equally to both resources under average precipitation (Lu et al. 2018). Yet soil moisture has direct effect on both plants and soil conditions. Increased soil water content can enhance soil N availability and N mineralization, leading to confounding effects on plant productivity (Wang et al. 2006).

In this study, I experimentally imposed drought to test the effect of soil moisture limitation on invasive plant soil-feedbacks and characteristics. I focused on *Microstegium vimineum* (Trin.) A. Camus, an invasive annual C_4 grass that has been shown to alter soil C and N cycles and establish self-reinforcing feedbacks by increasing turnover of fast-cycling organic carbon (Strickland et al. 2010), increasing rates of nitrification (Ehrenfeld et al. 2001, Kourtev et al. 2003, Lee et al. 2012) and partitioning greater amounts of N aboveground than native counterparts (Fraterrigo et al. 2011). *M. vimineum* also has a preference for establishing in wetter sites, but is capable of surviving a range of moisture conditions (Warren et al. 2011). I predicted that reductions in soil moisture imposed by rainout shelters would reduce resource-mediated feedbacks by limiting soil microbial decomposition and by physiologically constraining the productivity of *M. vimineum*. This would reduce quantity and quantity of detrital inputs to the soil, thereby weakening impacts of *M. vimineum* on soil C and N cycling.

Methods

Site Selection and Experimental Design

This study was conducted in the Central Hardwood Region of southern Illinois, USA, where *M. vimineum* has been a dominant understory invasive plant over two decades. I selected two sites to study within this region that have a close proximity and similar invasion history of invasion, but differing levels of N availability: Dixon Springs State Park (DSSP) and Giant City State Park (GCSP). DSSP (324 ha, 37°22' N, 88°39' W) has been invaded by *M. vimineum* since the mid-1990s. Land managers at this site have used prescribed fire every 3-6 years to manage woodlands to reduce fine fuel accumulation, promote oak-hickory regeneration, and stimulate the growth of native understory vegetation. GCSP (1,619 ha, 37°36' N, 89°11' W) is located ~65 km northwest of DSSP and has been invaded by *M. vimineum* since the early 2000s (Illinois Department of Natural Resources), and has not been managed with prescribed fire in its recent history. In a previous study, I found that DSSP tended to be more N limited than GCSP due to the differences in fire management history at each site (Rembelski and Fraterrigo, in prep.).

The region is characterized by a humid subtropical climate. Since 2005, mean temperatures at both sites averaged 24 °C in the summer (June-Aug) and 2 °C in the winter (Dec-Feb); annual precipitation totals averaged 135 cm for DSSP and 122 cm for GCSP (http://raws.dri.edu). Soils are classified as Alfisols (mesic Oxyaquic Fragiudalds) in the Grantsburg series at DSSP and the Hosmer series at GCSP. Both sites have a similar elevations (150-200 m), slopes (2-18%), and overstory communities, with the dominant species being elm (*Ulmus spp.*), maple (*Acer spp.*), ash (*Fraxinus spp.*), black walnut (*Juglans nigra*), shingle oak (*Quercus imbricaria*), sycamore (*Platanus occidentalis*), and sassafras (*Sassafras albidum*) (Appendix B).

I used a paired plot design to evaluate the effects of drought on *M. vimineum* populations and ecosystem processes. I established 12 pairs of 5 m x 5 m plots (n = 24 plots total) within invaded areas, including seven pairs at DSSP and five pairs at GCSP. Within each pair, plots were spaced at least 2 m apart to minimize environmental differences and assigned a treatment of either ambient or drought. I imposed drought by installing rainout shelters consisting of a 5 m x 5 m sheet of clear plastic greenhouse material at least 2 m above the surface of the plot, following the study design described in Refsland and Fraterrigo (2018). Rainout shelters were constructed using standard clear greenhouse film (Greenhouse Megastore, Danville, IL) with 91% light transmission to exclude precipitation from the soil while maintaining similar light conditions to its paired ambient plot. Rainout shelters were hung from nearby trees using plastic clips and rope from late May – November 2017.

Soil Respiration and Environmental Parameters

I installed two permanent 10-cm diameter PVC collars at each plot to measure soil respiration using a LI-COR 8100A portable infrared gas analyzer (LI-COR, Inc., Lincoln, NE). Measurements of soil CO_2 efflux were recorded twice monthly at each plot from June-November 2017, for a total of eleven measurements throughout the study period. Any new plant growth was removed from within the collars prior to taking measurements, but any surface litter was left intact within the collars. Flux measurements were always recorded in the afternoon between 10:00 and 16:00. Concurrently, I measured soil temperature (10-cm depth, Thermo Fisher Scientific, Waltham, MA) and volumetric soil moisture (7.5-cm depth, FieldScout TDR 100, Spectrum Technologies, Inc., Aurora, IL) adjacent to each collar. Additionally, I deployed data loggers (HOBO Micro Station H21 Data Loggers, Onset Computer Corporation, Bourne, MA)

paired with one soil temperature probe (10-cm depth) (S-TMB-M002 Temperature Sensor, Onset Computer Corporation) and one moisture probe (7.5-cm depth) (ECH_2O EC-5 Moisture Sensor, Decagon Devices Inc., Pullman, WA) at each site and recorded hourly values from June-November 2017. I used these continuous series of temperature and moisture to construct a continuous model of soil respiration at each plot.

Soil and Vegetation Sampling

Soils were sampled at each plot twice throughout the study period: once in July 2017 to characterize microbial biomass and enzyme activities, and again in August 2017 to test for C and N concentrations and pH. Soils were sampled using a 2.2-cm diameter soil probe to sample from 0-5 cm, 5-10 cm, and 10-15 cm soil depths below the mineral surface. Each sample represented a composite of four individual samples taken randomly across the plot. Fresh soils that were sampled in July were transported to the lab on ice and refrigerated at 4 ^{o}C and analyzed for microbial biomass. Subsamples of the fresh soils were frozen at -80 ^{o}C for four months prior to testing for extracellular enzyme activities. Soil samples collected in August were homogenized, air-dried, and sieved (< 2mm) prior to subsampling and testing for pH, fractionating for organic matter, and analyzing fractions for C and N. Bulk density was measured at each pair of plots using a standard 5-cm bulk density corer, while adjusting the weight and volume for any rocks or large roots.

Roots were also sampled at the same time as soils in August 2017 using a 3.5 cm PVC root corer. To determine standing root biomass, I collected three replicate cores from each plot separated at 0-5 cm, 5-10 cm, and 10-15 cm depths. Samples were transported to the lab on ice and frozen until processed. I processed root samples by washing the sample with deionized

water above a 53 μm sieve and thoroughly picking roots. Root samples were separated into fine (< 2 mm), coarse (2-5 mm), and very coarse (> 5mm) fragments and dried at 55 ^{o}C before weighing (Baer et al. 2010). Fine root samples were composited by plot across all three depths and ground prior to analyzing for C and N concentrations.

I measured aboveground *M. vimineum* biomass in each plot in August 2017 by clipping all stems at ground level within two 25 x 25 cm PVC quadrats randomly placed within the plot boundary. *M. vimineum* foliage was dried, ground, and homogenized before elemental and isotopic analysis for %C, %N, and δ^{13}C. I also quantified litter biomass by collecting a forest floor footprint from the same two 25 x 25 cm quadrats used to sample *M. vimineum*. Any litter above the top of the mineral soil was collected and dried at 60^{o}C until constant mass.

The overstory tree community was characterized for each pair of plots by identifying and quantifying diameter at breast height (DBH) of all adult trees (> 10 cm DBH) within 50 m of the plot center. I also measured photosynthetically active radiation (PAR/PDIFF) in June and July 2017 using a sample rate of 10 Hz and averaging four recordings across each plot (LI-191R Line Quantum Sensor, LI-COR, Inc., Lincoln, NE). I recorded four averaged PAR measurements at each plot by standing at the plot center and extending the light sensor to each of four corners of the plot. PAR recordings were subtracted from concurrent measurements of full light PAR to calculate the difference (PDIFF) at each plot.

Laboratory Analyses

Air-dried soil samples from August 2017 were analyzed for pH (2:1 mL H_2O:g soil) using a bench-top pH meter (Accumet AB15, Fisher Scientific, Waltham, MA) and total C and N and δ^{13}C of fractioned SOM using Costech 4010 CHNSO Elemental Analyzer (Costech

Analytical Technologies Inc., Valencia, CA, USA) interfaced with an isotope ratio mass spectrometer (Thermo Fisher Delta V Advantage, Fisher Scientific). Soils were fractionated into two pools of soil organic matter (SOM): (1) particulate organic matter (POM) and (2) mineral-associated organic matter (MAOM) as described in Bradford et al. (2008). I define the POM pool as any material larger than 53 μm in diameter and MAOM as any material smaller than 53 μm. POM contains the pool of faster-cycling organic matter mostly containing plant-derived particulates and materials, while the MAOM fraction is comprised of the slower-cycling organic carbon that is more microbially derived and processed (Schlesinger and Lichter 2001, Bradford et al. 2008). Briefly, I separated these fractions of organic matter using chemical dispersion with sodium hexametaphosphate (NaHMP) and physical separation above a 53 μm sieve. POM fractions were oven-dried at 115^{o}, while 130 mL subsamples of the MAOM fraction solution were oven-dried at 60 ^{o}C (Cambardella and Elliott 1992). Dried samples were ground and homogenized before analyzing for C and N during elemental analysis.

Fresh soil samples collected in July 2017 were used to measure active microbial biomass and microbial biomass C and N. Substrate induced respiration (SIR) was used to measure active microbial biomass as the pulse of CO_2 after the addition of a high quality substrate to fresh soil following the procedure described in Fierer and Schimel (2003). Throughout the 4-hour incubation period, I recorded headspace CO_2 concentrations throughout the incubation using a desktop infrared gas analyzer (LI-7000 CO_2/H_2O gas analyzer, LI-COR, Inc., Lincoln, NE). Simultaneous chloroform fumigation extractions (sCFE) were used to determine the concentrations of microbial biomass C and N using the method described by Fierer and Schimel (2003). Briefly, I combined pairs of fresh soil samples with 0.5 M K_2SO_4, while treating only one sample from each pair with 0.5 mL EtOH-free chloroform. Dissolved organic C and N were

determined from the extracts using a TOC-L and TNM-L analyzer (Shimadzu Corporation, Kyoto, Japan). No correction factor was applied to the reported values for sCFE and SIR.

I prepared assays to determine activities of four extracellular soil enzyme activities: β-glucosidase (BG), β-N-acetylglucosaminidase (NAG), polyphenol oxidase (PPO), and peroxidase (PER). These enzymes were selected for their ecological relevance and role in decomposition of SOM. BG and NAG are hydrolytic enzymes primarily involved in the breakdown of fast-cycling organic matter, where BG primarily functions to hydrolyze cellulose into glucose (Ljungdahl and Eriksson 1985), while NAG is associated with N acquisition and the breakdown of chitin (Sinsabaugh et al. 2008). PPO and PER are lignolytic enzymes that are more important for the cycling of complex and recalcitrant SOM (Weintraub et al. 2007). I used the procedure from Finzi et al. (2006) to prepare the assays. To summarize, soils were suspended using sodium acetate buffer solution and added to 96-well plates containing wells for the soil slurry, quench wells with 4-methylumbelliferyl, or substrate solution. Assays were incubated and measured using a microplate reader (BioTek Synergy HT Multi-Mode Microplate Reader, BioTek Instruments, Inc., Winooski, VT). Fluorimetric assays were read at 365 nm excitation and 460 nm emission and absorbance assays were read at 460 nm absorbance. Enzyme activities are reported here as the amount of substrate cleaved per mass soil during the incubation time.

Data Analysis

I calculated a continuous series of hourly soil temperature, moisture, and CO_2 efflux for each plot by using the continuous datasets of soil temperature and moisture for the plot's respective site. First, I filled any gaps in the continuous site-level data series by using simple

linear regression against datasets from the Illinois State Water Survey (ISWS) stations (Water and Atmospheric Resources Monitoring Program, Illinois Climate Network, Illinois State Water Survey, Champaign, IL). The ISWS provided me with two continuous datasets from climate monitoring sites at DSSP and Carbondale, IL (10 miles north of GCSP), each consisting of hourly soil temperature under sod (10 cm depth) and soil moisture (avg. between 5 and 10 cm depth). Point measurements of flux, soil moisture, and soil temperature at both collars were averaged by plot before analyzing. Next, I matched point measurements of soil respiration, temperature, and moisture to the corresponding date and hour within the continuous site-level dataset. I used simple linear regression between the point measurements and the continuous datasets to derive an hourly series of soil moisture and temperature values for all 40 plots. These continuous datasets of soil temperature and moisture were used to derive the hourly soil CO_2 fluxes at each plot using the following natural log-linear quadratic model adapted from Martin and Bolstad (2005):

$$\ln(R_s) = b_0 + b_1(SWC) + b_2(SWC^2) + b_3(soilT) + b_4(soilT^2) + b_5(soilT * SWC)$$

where R_s is soil respiration, *SWC* is volumetric soil water content, and *soilT* is soil temperature. I used this equation because of the unimodal response of soil respiration to variation in moisture and temperature, while the natural logarithmic transformation of respiration corrects for any violation of homogeneity of variance (Bowden et al. 1998). Beta coefficients were derived for each plot using point measurements of soil respiration, soil temperature, and soil moisture, and were used to predict a series of hourly soil respiration at each plot from June-November 2017

(Appendix C). Fluxes were converted from μmol CO_2 m^{-2} s^{-1} to g C m^{-2} hr^{-1}, and summed to determine cumulative flux per month.

I used linear mixed effects models in to evaluate the effects of drought on *M. vimineum* and soil properties. I included treatment (drought or ambient), site (DSSP or GCSP), soil depth (0-5 cm, 5-10 cm, 10-15 cm) and all possible interactions as fixed effects in the model, while including plot pair as a random effect to account for spatial heterogeneity among pairs. Response variables were log transformed when necessary to meet the assumptions of normality and homogeneity of variance. I used the Kolmogorov-Smirnov test to confirm a normal distribution of data and Levene's test for heteroscedasticity. When significant interactions were detected, I performed a post-hoc contrast of least squared means using Tukey's HSD correction for multiple comparisons. Results were considered significant if $p < 0.05$ or marginally significant if $p < 0.10$. All analyses were performed in R (R Core Development Team, 2018) using the "lme4" and "lsmeans" packages.

Results

Microclimate and Soil Respiration

The rainout shelters decreased the moisture content of surface soils (0-7.5 cm depth) by an average of 10.4% v/v ($F_{1,10} = 125.52$, $p < 0.01$). Reductions in soil moisture depended on site ($F_{1,10} = 7.62$, $p = 0.02$), with average soil moisture from June - November reduced from 25.2 to 17.4 ± 1.4% (SE) at DSSP and from 28.1 to 15.1 ± 1.6% at GCSP (Fig. 3.1). The effect of drought treatment on soil pH varied with site ($F_{1,45} = 8.09$, $p < 0.01$), with drought increasing pH compared to ambient plots at DSSP from 5.33 to 5.52 and decreasing pH at GCSP from 5.73 to 5.56. Drought treatment had no effect on light availability as measured by PDIFF.

Modeled cumulative soil respiration from June-November 2017 varied significantly by treatment ($F_{1,10} = 12.06$, $p < 0.01$). Drought treatment decreased cumulative soil CO_2 release by 24% on average compared to ambient plots (Fig. 3.2). When analyzed by month, I found significant differences between treatments during the months of July ($F_{1,10} = 34.14$, $p < 0.01$), August ($F_{1,10} = 21.52$, $p < 0.01$), and September ($F_{1,10} = 30.33$, $p < 0.01$).

M. vimineum Shoot and Root Biomass

Drought treatment decreased aboveground *M. vimineum* biomass by 33% compared to ambient plots ($F_{1,10} = 1.27$, $p = 0.02$) (Fig. 3.3). *M. vimineum* leaf C:N ratios differed by site ($F_{1,10} = 6.02$, $p = 0.03$), treatment ($F_{1,10} = 13.05$, $p < 0.01$), and the interaction between site and treatment ($F_{1,10} = 10.26$, $p < 0.01$). Reduced soil moisture significantly increased *M. vimineum* leaf C:N from an average of 15.5 to 19.0 ± 0.7 (SE) at GCSP ($p < 0.01$), but had no significant impact on leaf C:N at DSSP where leaf C:N ratios increased from 19.0 to 19.2 ± 0.6 under the drought treatment (Fig. 3.3).

Fine root biomass did not differ by treatment, but varied marginally by the interaction between site and soil depth ($F_{2,60} = 2.46$, $p = 0.09$). Site-level differences in fine root biomass were greatest in the surface 0-5 cm soil layer, where DSSP had 117% more fine root biomass than GCSP ($p = 0.08$) (Fig. 3.4). Drought treatment effects on fine root N concentration varied with site ($F_{1,20} = 5.85$, $p = 0.03$). Compared to ambient plots, root N concentration increased by 20% at DSSP, and did not change at GCSP. This led to a marginally significant interaction between site and treatment for root C:N ratios ($F_{1,10} = 3.81$, $p = 0.07$). Drought treatment significantly reduced average fine root C:N ratio from 38.3 to 32.4 ± 1.73 at DSSP ($p = 0.03$), and had no effect at GCSP (Fig. 3.5).

Mineral Soil C and N Stocks

Neither treatment nor site had a significant effect on soil C and N stocks in either the MAOM or POM fractions of SOM (Table 3.1). However, drought treatment led to significant declines in the POM C:N ratios ($F_{1,45} = 4.29$, $p = 0.04$) (Fig. 3.6).

Soil concentrations of DOC were 55% higher at DSSP compared to GCSP ($F_{1,9} = 6.08$, $p = 0.04$). Drought treatment significantly altered DON concentrations ($F_{1,45} = 4.62$, $p = 0.04$) by decreasing DON by an average of 12% (Table 3.1).

Soil Microbial Biomass ad Extracellular Enzyme Activities

Active microbial biomass differed by the interaction between site and depth ($F_{2,50} = 2.46$, $p = 0.01$). Active microbial biomass was 56% higher at DSSP than GCSP in the surface 0-5 cm soil layer, and was 19% higher at GCSP than DSSP in the deepest 10-15 cm soil layer (Table 3.2). Concentrations of microbial biomass N, as measured by sCFE, differed marginally by site. Microbial biomass N was 31% higher at GCSP compared to DSSP ($F_{1,10} = 4.01$, $p = 0.07$). Consequently, microbial biomass C:N ratios were significantly lower at GCSP (3.7 ± 0.36 SE) compared to DSSP (5.00 ± 0.41; $F_{1,10} = 5.53$, $p = 0.04$) (Table 3.2).

Activity of the extracellular enzyme BG differed by treatment, with drought increasing BG activity by 32% compared to ambient ($F_{1,50} = 11.19$, $p < 0.01$; Table 3.2). NAG activity was not affected by treatment or site. PPO activity varied by treatment ($F_{1,35} = 4.81$, $p = 0.04$) and the interaction between site and soil depth ($F_{1,35} = 4.60$, $p = 0.02$). Drought decreased PPO activity by 16% compared to ambient, and PPO activity was significantly higher at GCSP in the deepest 10-15 cm soil layer ($p < 0.01$). PER activity varied by the interaction between site and

on soil depth ($F_{1,35} = 6.56$, $p < 0.01$) but was not affected by treatment. PER activity was significantly higher at GCSP compared to DSSP in both the 5-10 cm ($p = 0.03$) and 10-15 cm soil layers ($p < 0.01$).

Discussion

I evaluated the effects of soil moisture limitation on invasive plant-soil feedbacks and soil C and N cycling by experimentally inducing drought conditions in plots invaded by the non-native grass *M. vimineum*. I found that reductions in soil moisture led to significant declines in *M. vimineum* biomass, soil respiration, POM-C:N ratios, and concentrations of DON. Collectively, these results suggest that soil moisture limitation may weaken the positive resource-mediated feedbacks of invasion both directly by limiting productivity and indirectly by slowing microbial decomposition and decreasing soil N availability.

In support of my hypothesis, I found that reducing soil moisture had a significant negative effect on soil respiration and aboveground biomass of *M. vimineum*, suggesting a direct influence of moisture on productivity and soil microbial processes. Because I found no evidence of changes in fine root distribution between treatments, I primarily attributed the decrease in soil respiration to reduced microbial metabolic activity. I also found that activity of the enzyme BG is higher in plots with reduced soil moisture, though this is likely an indicator of abiotic stress, as microbes attempt to mobilize resources by producing energy-acquiring enzymes (Schimel and Weintraub 2003).

The observed declines in *M. vimineum* productivity under drought are likely the result of both water stress on the plant and declines in N availability, where soil moisture is a limiting variable on both primary productivity and microbial turnover of soil organic matter (Harper et al.

2005, McCulley et al. 2005). My data suggest that rates of decomposition are being reduced at plots under drought conditions, indicating a shift in microbial composition and functioning (Tiemann and Billings 2011). The soil microbial community is also shown to alter its physiology in respond to drought in ways that can impact soil N cycling, as microorganisms acquire N-rich protective osmolytes to protect themselves against strongly negative soil osmotic potentials (Schimel et al. 2007, Borken and Matzner 2009). Although I cannot directly determine the relative contribution of moisture limitation and N limitation on plant growth, I can use previously established site-level differences in N status to better extrapolate the relative effect of each variable on *M. vimineum*.

In a related study, I found that differences in N availability between the two study sites were due to contrasting fire management history of each site (Rembelski and Fraterrigo, in prep). Here, I find comparable evidence suggesting that productivity at DSSP is more N limited than at GCSP. In line with this finding, I observed that site modulated the effect of drought on the leaf chemistry of *M. vimineum*. At DSSP, the site that is more N limited, there was no difference in *M. vimineum* leaf C:N ratio between drought and ambient treatments. At GCSP, however, drought treatment resulted in a significant increase in *M. vimineum* leaf C:N ratio compared to the ambient treatment (Fig. 3.3). This effect suggests a strong coupling between soil moisture and soil N for regulating plant growth. This effect may have manifested only at the GCSP site because moisture limitation could have negatively affected the physiology of plant roots to limit uptake of N, or by limiting conversion of organic N into plant-available forms by soil microbes.

Despite substantial changes in *M. vimineum* biomass and foliage chemistry under drought conditions, I found little evidence for shifting C and N stocks in the soil. The only significant effect detected on soil organic matter was a shift toward lower POM-C:N ratios and DON

concentrations under drought conditions. These effects may be due to decreased turnover of SOM and reduced N mineralization as a direct effect of moisture limitation (Paul et al. 2003, Wang et al. 2006). Longer-term studies will be required to determine whether moisture mediated changes to invader impacts lead to altered soil carbon and nutrient stocks over time.

This study, however, only focused on the short-term effects of moisture limitation on nonnative grass invasion and does not address the effects of an increase in available moisture following a period of prolonged drought. Despite this, studies suggest that the pulse of net C and N mineralization following a pulse of soil moisture after prolonged drying is unlikely to fully compensate for the lack of mineralization occurring during drought (Borken and Matzner 2009). This suggests that the effects of drought on invader impacts are likely to last beyond the period of drought recovery. Going forward, studies should aim to characterize long-term responses of invasion to repeated and prolonged moisture stress, particularly in the context of altering dynamics of resource-mediated invasive plant feedbacks.

My study provides context for how the effects of invasion could be impacted by altered moisture conditions. Over longer timescales, the resource-mediated feedbacks of plant invasions may weaken as resource availability changes over time (Yelenik and D'Antonio 2013, Flory et al. 2017). Yet changes in precipitation and moisture patterns due to climate change are likely to alter the nutrient dynamics of invaded landscapes at increasingly higher rates, suggesting the importance of considering the impacts of these disturbances as they co-occur. By using experimental field methods to isolate the individual effects of climate change on invader impacts, this study provides insight into how drivers of global change may affect ecosystem functioning in the future.

Figures and Tables

Figure 3.1 Measured and average volumetric soil water content (%) at invaded study sites DSSP and GCSP from June – November 2017. Soil moisture was measured to a soil depth of 7.5 cm below the mineral surface. Drought was experimentally imposed with rainout shelters to exclude precipitation from late May – November 2017.

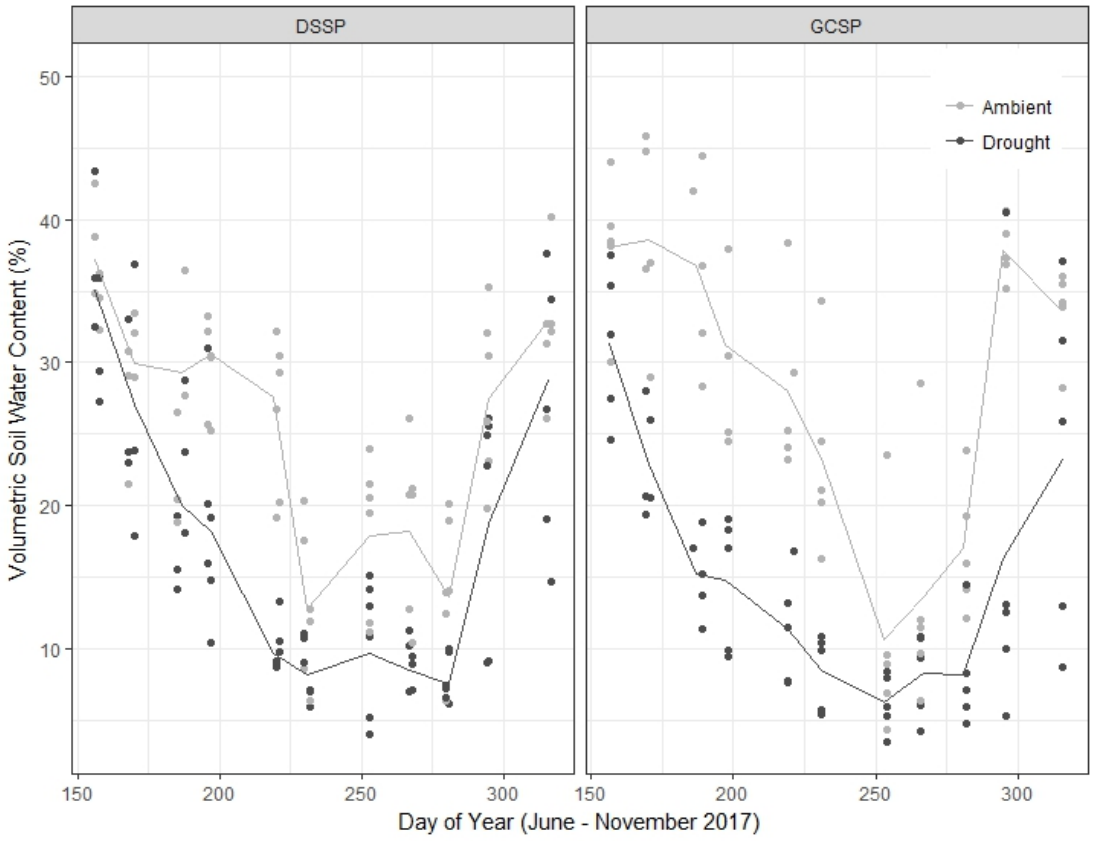

Figure 3.2 Modeled cumulative soil respiration by month (g C m^{-2} $month^{-1}$; mean ± 1 SE) (A), and by the entire study period from June-November 2017 (g C m^{-2}; mean ± 1 SE) (B). Results are separated by site (DSSP or GCSP) and drought treatment. Cumulative respiration was modeled using a log-linear quadratic model based on hourly soil temperature and soil moisture data from June-November 2017 († $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

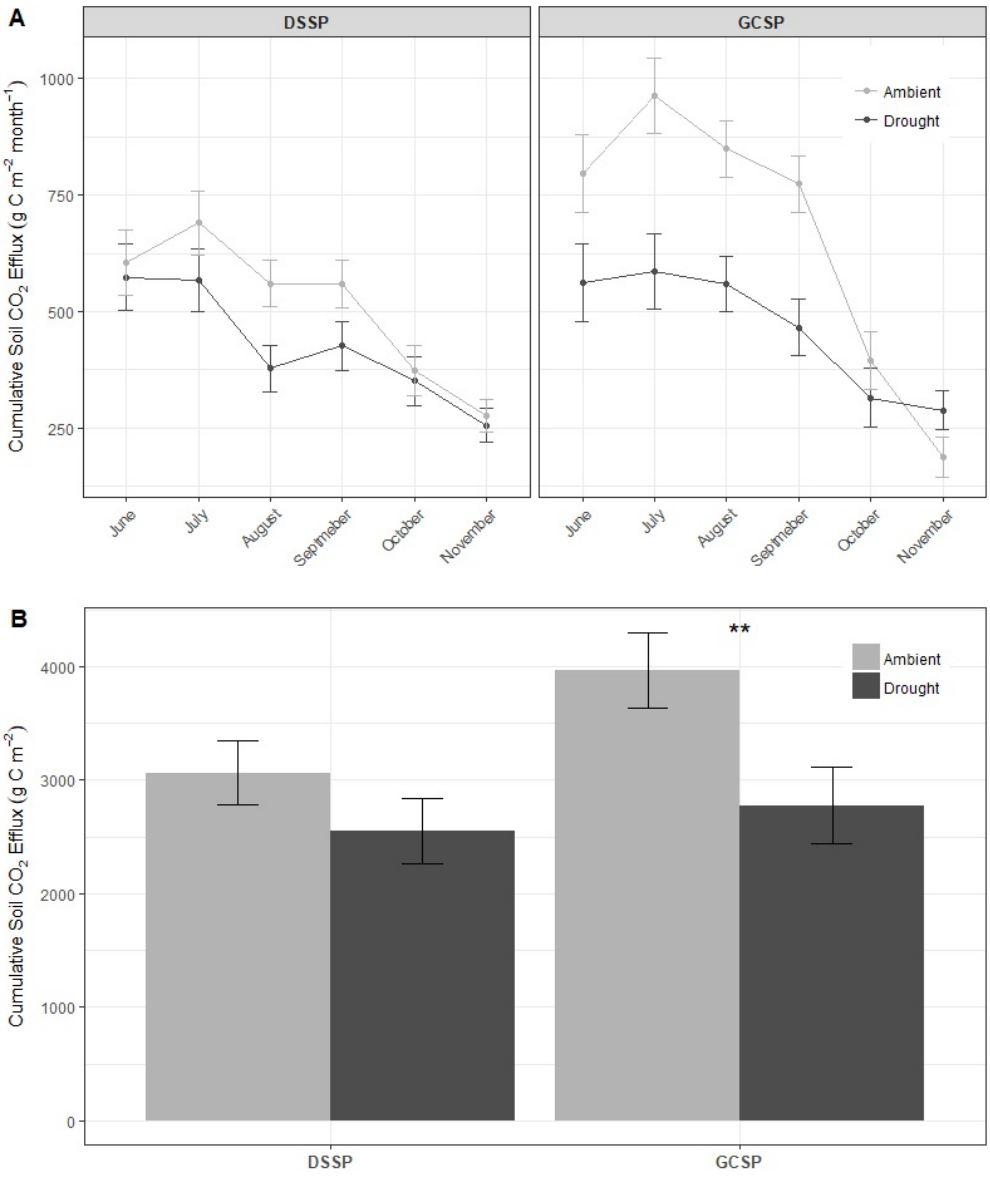

Figure 3.3 Aboveground *M. vimineum* biomass (g m^{-2}; mean ± 1 SE) (A) and C:N of *M. vimineum* foliage (B) at invaded study sites DSSP and GCSP in August 2017. Drought was experimentally imposed with rainout shelters to exclude precipitation from late May – November 2017. Values are presented as dry weight biomass after oven dried to constant mass

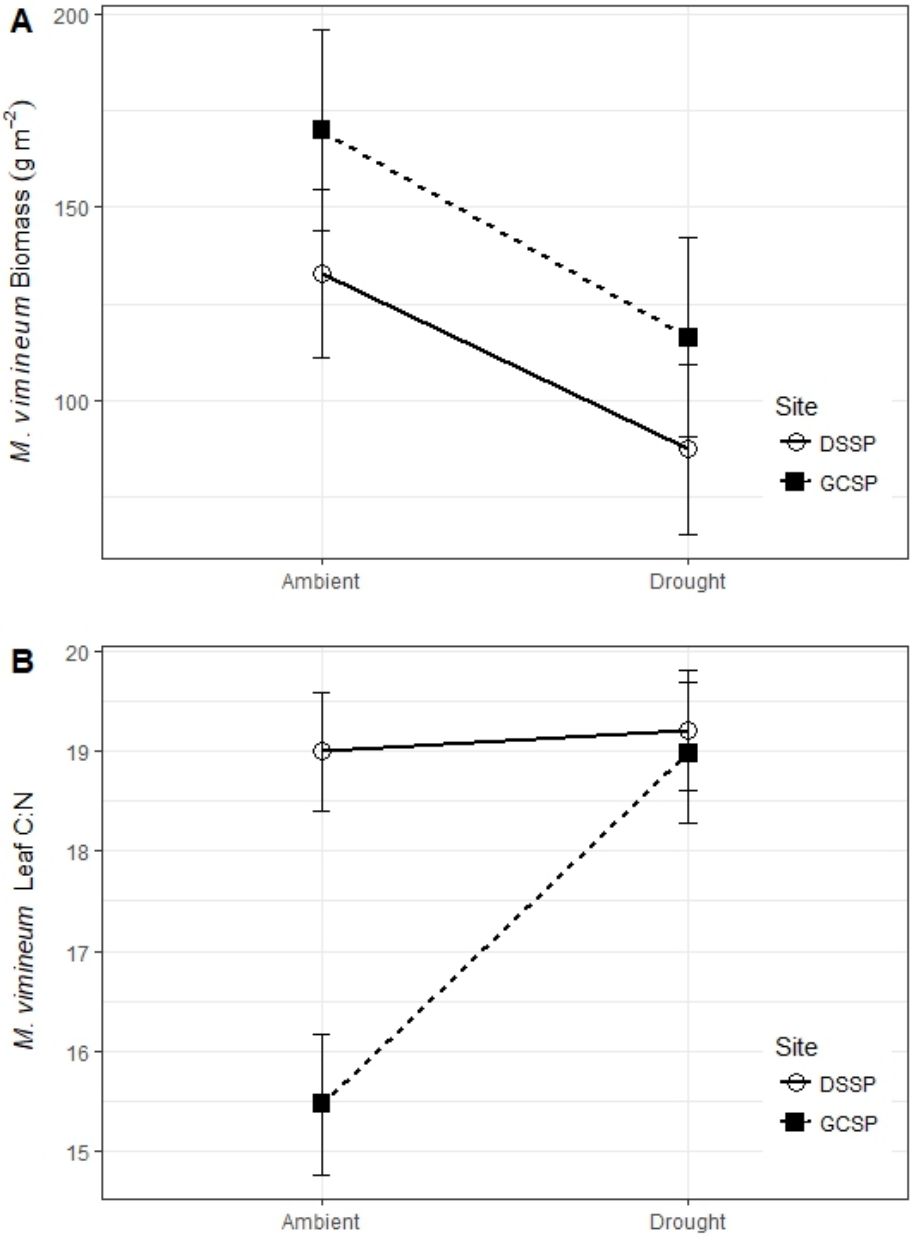

Figure 3.4 Fine root biomass (g m^{-2}; mean ± 1 SE) at DSSP and GCSP sites separated by drought treatment and mineral soil depths of 0-5 cm, 5-10 cm, and 10-15 cm from the surface. Fine roots are classified as roots with a diameter < 2 mm after drying. Roots were sampled in August 2017 and oven dried to constant mass after separating and washing from soil.

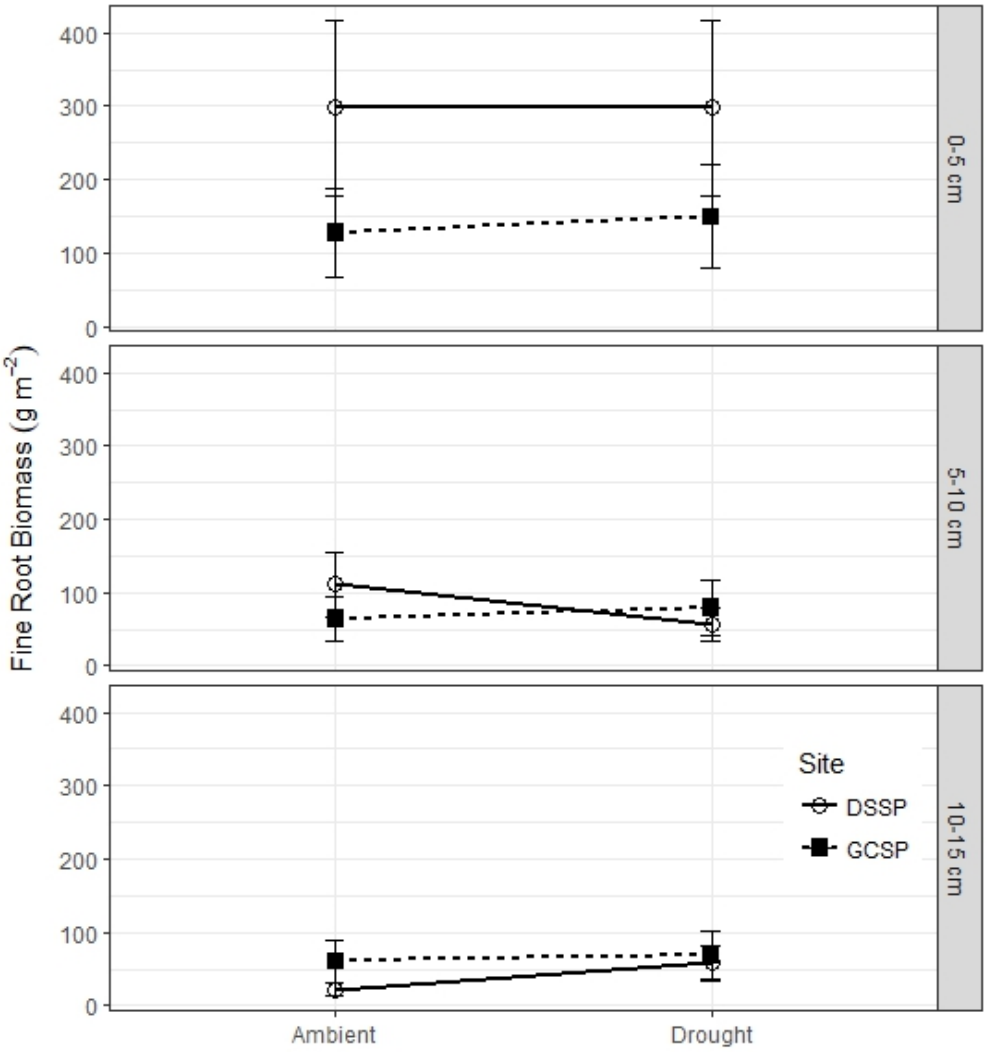

Figure 3.5 Fine root biomass C:N ratios in plots invaded by *M. vimineum* at study sites DSSP and GCSP in August 2017.

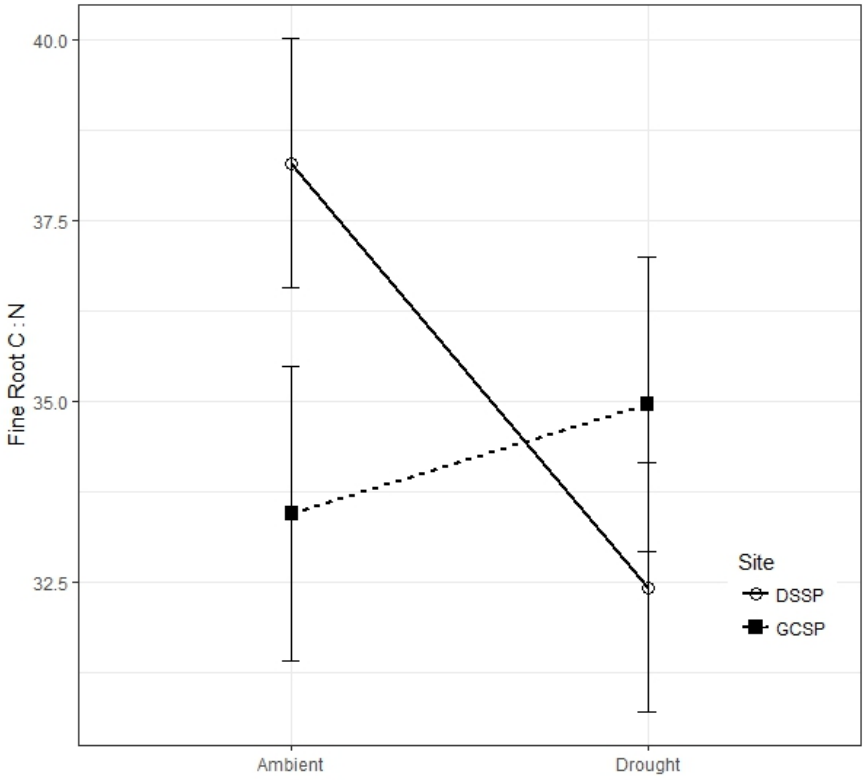

Figure 3.6 C:N ratios of fractionated soil particulate organic matter (POM) and mineral-associated organic matter (MAOM). Data are presented as atomic ratio means (± 1 SE) at sites GCSP and DSSP under ambient or drought treatment.

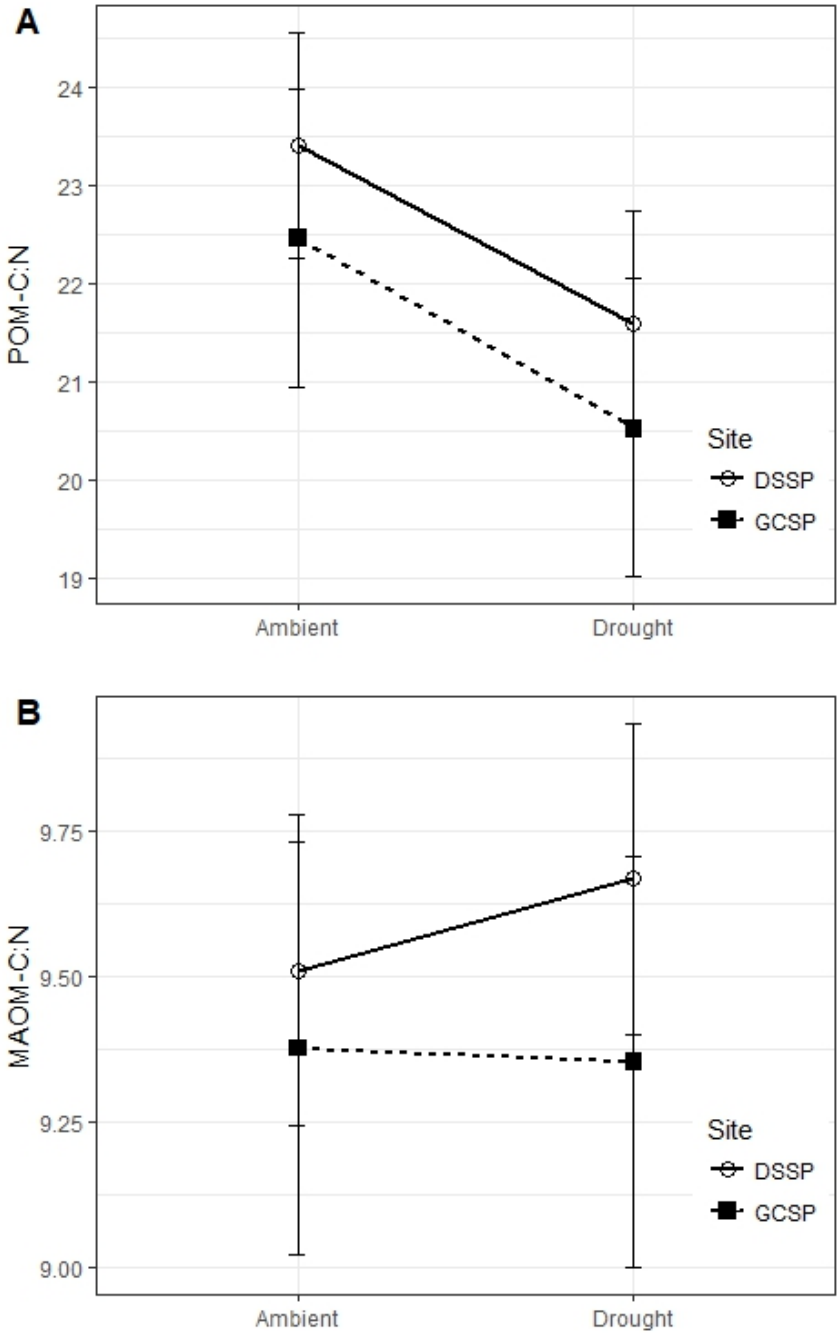

Table 3.1 Mineral soil C and N stocks of fractionated soil particulate organic matter (POM) and mineral-associated organic matter (MAOM), dissolved organic carbon (DOC) concentrations, and dissolved organic nitrogen (DON) concentrations. Significant main effects or interactions from the linear mixed effects model are indicated by asterisks († $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Site:		DSSP		GCSP		Significant Effects:					
Treatment:		Ambient	Drought	Ambient	Drought	*Site*	*Treatment*	*Depth*	*Site x Treatment*	*Site x Depth*	*Site x Treatment x Depth*
Soil pH	0-5 cm	5.51 ± 0.14	5.64 ± 0.14	5.86 ± 0.20	5.53 ± 0.19			*	**		
	5-10 cm	5.30 ± 0.14	5.56 ± 0.14	5.68 ± 0.19	5.54 ± 0.19						
	10-15 cm	5.16 ± 0.13	5.32 ± 0.14	5.64 ± 0.19	5.57 ± 0.19						
POM-C ($g\ C\ m^{-2}$)	0-5 cm	732.08 ± 88.74	633.86 ± 76.83	615.56 ± 98.7	751.97 ± 120.58			***			
	5-10 cm	172.28 ± 20.88	154.22 ± 18.69	161.75 ± 25.94	153.44 ± 24.60						
	10-15 cm	78.12 ± 9.47	76.01 ± 9.21	76.09 ± 12.20	83.23 ± 13.35						
POM-N ($g\ N\ m^{-2}$)	0-5 cm	35.32 ± 4.54	34.94 ± 4.49	30.61 ± 4.66	35.94 ± 5.47			***			
	5-10 cm	7.72 ± 0.99	7.70 ± 0.99	8.49 ± 1.29	9.06 ± 1.38						
	10-15 cm	3.03 ± 0.39	2.89 ± 0.37	3.76 ± 0.57	3.84 ± 0.58						
POM C:N (*atomic ratio*)	0-5 cm	20.73 ± 1.40	18.14 ± 1.23	20.84 ± 1.87	19.92 ± 1.78		*	***			
	5-10 cm	22.33 ± 1.51	20.02 ± 1.36	20.81 ± 1.86	18.56 ± 1.66						
	10-15 cm	25.82 ± 1.75	26.28 ± 1.78	24.29 ± 2.17	22.56 ± 2.02						
MAOM-C ($g\ C\ m^{-2}$)	0-5 cm	996.08 ± 64.55	1103.05 ± 71.49	980.68 ± 84.08	1100.69 ± 94.37			***			
	5-10 cm	666.14 ± 43.17	704.89 ± 45.68	733.88 ± 62.92	769.61 ± 65.98						
	10-15 cm	516.05 ± 33.44	491.35 ± 31.84	478.19 ± 41.00	511.60 ± 43.86						
MAOM-N ($g\ N\ m^{-2}$)	0-5 cm	97.53 ± 6.09	105.97 ± 6.62	103.26 ± 7.63	109.04 ± 8.06			***			
	5-10 cm	71.50 ± 4.46	74.90 ± 4.68	84.39 ± 6.24	88.80 ± 6.56						
	10-15 cm	57.70 ± 3.60	53.80 ± 3.36	57.98 ± 4.28	59.89 ± 4.43						
MAOM C:N (*atomic ratio*)	0-5 cm	10.21 ± 0.30	10.41 ± 0.31	9.99 ± 0.39	10.07 ± 0.40			***			
	5-10 cm	9.32 ± 0.28	9.41 ± 0.28	9.31 ± 0.37	9.18 ± 0.36						
	10-15 cm	8.94 ± 0.27	9.13 ± 0.27	8.73 ± 0.34	8.70 ± 0.34						
DOC ($\mu g\ C\ g\ soil^{-1}$)	0-5 cm	110.03 ± 19.58	100.03 ± 17.80	84.29 ± 19.84	83.10 ± 19.56	*		***			
	5-10 cm	66.09 ± 11.76	69.39 ± 12.35	49.15 ± 11.57	45.69 ± 10.75						
	10-15 cm	82.46 ± 14.67	76.98 ± 13.70	42.73 ± 10.06	34.46 ± 8.11						
DON ($\mu g\ C\ g\ soil^{-1}$)	0-5 cm	23.50 ± 3.55	25.19 ± 3.80	33.53 ± 5.54	26.06 ± 4.31		*	***			
	5-10 cm	14.56 ± 2.20	10.80 ± 1.63	14.55 ± 2.40	13.49 ± 2.23						
	10-15 cm	8.19 ± 1.24	8.66 ± 1.31	10.51 ± 1.74	7.99 ± 1.32						

Table 3.2 Microbial Biomass C and N concentrations measured by simultaneous chloroform fumigation extractions (sCFE), active microbial biomass measured by SIR, and soil enzyme activities (mean ± 1 SE). Significant main effects or interactions from the linear mixed effects models are indicated by asterisks († $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Site:		DSSP		GCSP		Significant Effects:				
Treatment:		Ambient	Drought	Ambient	Drought	*Site*	*Treatment*	*Depth*	*Site x Treatment*	*Site x Depth*	*Site x Treatment x Depth*
Microbial Biomass-C ($\mu g\ C\ g\ soil^{-1}$)	0-5 cm	156.83 ± 39.58	143.92 ± 36.32	142.72 ± 42.62	139.26 ± 41.59			***			
	5-10 cm	64.46 ± 16.27	68.86 ± 17.38	74.51 ± 22.25	63.77 ± 19.04						
	10-15 cm	25.04 ± 6.32	30.49 ± 7.69	20.16 ± 6.02	33.95 ± 10.14						
Microbial Biomass-N ($\mu g\ N\ g\ soil^{-1}$)	0-5 cm	32.10 ± 5.66	30.06 ± 5.30	44.05 ± 9.19	33.67 ± 7.03	†		***			
	5-10 cm	12.03 ± 2.12	13.98 ± 2.47	16.52 ± 3.45	14.40 ± 3.01						
	10-15 cm	5.80 ± 1.02	5.21 ± 0.92	7.55 ± 1.58	9.31 ± 1.94						
Microbial Biomass C:N (atomic ratio)	0-5 cm	4.89 ± 0.73	4.79 ± 0.72	3.24 ± 0.58	4.14 ± 0.74	*					
	5-10 cm	5.36 ± 0.81	4.93 ± 0.74	4.51 ± 0.80	4.43 ± 0.79						
	10-15 cm	4.32 ± 0.65	5.85 ± 0.88	2.67 ± 0.47	3.65 ± 0.65						
Active Microbial Biomass ($\mu g\ CO_2\text{-}C\ g\ soil^{-1}\ hr^{-1}$)	0-5 cm	31.14 ± 8.90	32.92 ± 9.41	23.24 ± 7.86	18.23 ± 6.16			***		*	
	5-10 cm	8.42 ± 2.41	9.75 ± 2.79	8.71 ± 2.95	8.27 ± 2.80						
	10-15 cm	3.85 ± 1.10	4.68 ± 1.34	4.88 ± 1.65	5.26 ± 1.78						
β-Glucosidase (BG) Activity ($nmol\ g\ soil^{-1}\ hr^{-1}$)	0-5 cm	0.091 ± 0.016	0.120 ± 0.020	0.101 ± 0.020	0.123 ± 0.025		**	***		†	
	5-10 cm	0.024 ± 0.004	0.034 ± 0.006	0.035 ± 0.007	0.053 ± 0.011						
	10-15 cm	0.014 ± 0.002	0.016 ± 0.003	0.021 ± 0.004	0.029 ± 0.006						
β-N-Acetylglucosaminidase (NAG) Activity ($nmol\ g\ soil^{-1}\ hr^{-1}$)	0-5 cm	0.043 ± 0.008	0.041 ± 0.008	0.052 ± 0.011	0.040 ± 0.009			***			
	5-10 cm	0.019 ± 0.004	0.014 ± 0.003	0.018 ± 0.004	0.018 ± 0.004						
	10-15 cm	0.012 ± 0.002	0.008 ± 0.002	0.011 ± 0.002	0.012 ± 0.003						
Phenol Oxidase (PPO) Activity ($nmol\ g\ soil^{-1}\ hr^{-1}$)	0-5 cm	0.743 ± 0.118	0.562 ± 0.089	0.652 ± 0.116	0.565 ± 0.100	†	*			*	
	5-10 cm	0.575 ± 0.091	0.543 ± 0.086	0.764 ± 0.136	0.692 ± 0.123						
	10-15 cm	0.558 ± 0.089	0.341 ± 0.054	0.804 ± 0.143	0.727 ± 0.129						
Peroxidase (PER) Activity ($nmol\ g\ soil^{-1}\ hr^{-1}$)	0-5 cm	1.332 ± 0.170	1.204 ± 0.154	1.269 ± 0.181	1.229 ± 0.175	*		*		**	
	5-10 cm	0.869 ± 0.111	0.928 ± 0.118	1.289 ± 0.184	1.283 ± 0.183						
	10-15 cm	0.895 ± 0.114	0.653 ± 0.083	1.421 ± 0.203	1.254 ± 0.179						

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CHAPTER 4: SYNTHESIS OF DATA CHAPTERS

Synthesis of Chapter 2 and Chapter 3

The two studies presented in Chapters 2 and 3 provide insight into how different forms of climate-mediated disturbance may alter the self-reinforcing feedbacks of plant invasions. While Chapter 2 focuses on how fire-mediated feedbacks can influence the biogeochemical impacts of a non-native grass, Chapter 3 focuses specifically on the isolated effects of drought on invasion feedbacks. I chose to focus my thesis on the impacts of either fire or drought on invasion because of their importance in understanding some of the most pertinent aspects of global change. I also chose to focus on *Microstegium vimineum* invasion in temperate deciduous forests for both chapters, as this species is a widespread and persistent invader across eastern hardwood forests. As the ecological integrity of forests becomes increasingly threatened by climate disturbance and changing fire regimes (Millar and Stephenson 2015), it becomes more important to understand how plant invasions may respond.

Fire and drought are inherently linked as ecosystem stressors, and fire regimes can change in response to increased frequency and duration of drought events (Falk et al. 2007, Pausas and Fernandez-Munoz 2012). Decreases in soil and fuel moisture from drought can lead to increases in fire severity (Certini 2005), while mineral soil moisture content can mediate the effects of fire on soil biological properties (Choromanska and DeLuca 2002). Fire may also exacerbate the effects of drought by eliminating forest floor litter, leading to greater exposure of mineral soil, higher evaporation, and reduced soil moisture (Neary et al. 1999). These stressors can have substantial effects on invasive plants, especially non-native grasses that can recover from fire at a faster rate than native species (D'Antonio and Vitousek 1992), and C_4 species that utilize a higher water use efficiency pathway (Sage and Kubien 2003). My thesis project aims to

study these disturbances individually to determine their main effects on non-native grass invasion. By observing the impacts of fire and drought separately, I could examine the effects of fire, while having additional data to help determine which effects of fire may have resulted from changes in soil moisture.

My thesis also focused on the additional effect of fire legacy by conducting research across two invaded sites with contrasting fire management history, as recent studies have shown past fire frequency to have substantial impacts on soil microbial communities (Miesel et al. 2012) and soil carbon (C) and nitrogen (N) dynamics (Toberman et al. 2014, Pellegrini et al. 2018). By conducting the study across sites with a history of repeated prescribed burning or fire suppression, I could evaluate the importance of fire history for mediating the effects of burning or drought. In Chapter 2, I found that the effect of burn treatment was usually dependent on the fire history of the site. The results from Chapter 2 provided support for the hypothesis that the site with a history of repeated burning was more N limited than the site without a history of burning. These data from Chapter 2 provided deeper insight for determining the combined effects of N limitation and drought in Chapter 3, where I found that changes in *M. vimineum* chemistry after drought was largely mediated by site.

Limitations

Both studies presented in this thesis are limited by the inherent imperfections of the study design and by the environmental variables that were not directly measured. It is impossible to fully distinguish the differences between study sites based entirely on their fire histories. While these sites are characterized by overwhelming similarities, they are also contrasted by differences in land use history, annual precipitation, and variable soil conditions. These studies are also

limited by the lack of data on fire intensity, including temperature and flame height, as well as a direct measure of nitrogen availability. These studies were also extremely limited in length. Both studies were conducted over the timeframe of a single growing season, so any long-term changes or recoveries from disturbance were not observed or recorded. In future research on these topics, studies should aim to focus on additional sites with long-term records of fire history, while directly measuring the effects on soil nitrogen availability.

Conclusions

The two data chapters in this thesis provide complementary results that address how the direct and indirect effects of climate change may weaken the self-reinforcing feedbacks of non-native plant invasions. By conducting an experimental field study that isolated the effects of fire and drought on an invasive grass, I quantified how repeated fire may weaken invasive plant-soil feedbacks through increased ecosystem N loss, and how drought may have a similar effect by indirectly reducing N availability. Both of these studies enhance understanding for how invasive plant-soil feedbacks may change over time, especially in response to future global change.

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APPENDIX A: SITE DESCRIPTIONS

	Dixon Springs State Park (DSSP)	**Giant City State Park (GCSP)**
Geographic Coordinates	37°36' N, 89°11' W	37°22' N, 88°39' W
Area	324 ha	1,619 ha
Fire History (since 1990s)	Burned every 3-6 years	First woodland burn in 2016
M. vimineum Invasion History	Since mid-1990s	Since early 2000s
Summer Temperature (avg. since 2005)	24°C	24°C
Winter Temperature (avg. since 2005)	2°C	2°C
Annual Precipitation (avg. since 2005)	135 cm	122 cm
Elevation	150-200 m	150-200 m
Soils	fine-silty, mixed, active, mesic Oxyaquic Fragiudalfs in the Grantsburg series, with one pair of plots (pair XI) characterized as Ultic Hapludalfs in the Wellston series	fine-silty, mixed, active, mesic Oxyaquic Fragiudalfs in the Hosmer series, with one plot pair (pair III) classified as a coarse-silty, mixed, active, acid, mesic Fluventic Endoaquept in the Belknap series
Land Use History	Mixture of mature secondary oak-history forest and early successional woodlands established after abandonment from agriculture in the 1960s	Preserved as a natural recreation area after being established as a state park in 1927
Dominant Overstory Community	Elm (*Ulmus spp.*), Sugar Maple (*Acer saccharum*), Ash (*Fraxinus spp.*), Black Walnut (*Juglans nigra*), Persimmon (*Diospyros virginiana*), Eastern Red Cedar (*Juniperus virginiana*), Sassafras (*Sassafras albidum*), Red Maple (*Acer rubrum*), and Hackberry (*Celtis occidentalis*)	Box elder (*Acer negundo*), Elm (*Ulmus sp.*), Shingle oak (*Quercus imbricaria*), Sycamore (*Platanus occidentalis*), Sugar Maple (*Acer saccharum*), Black Walnut (*Juglans nigra*), and Sassafras (*Sassafras albidum*)

APPENDIX B: OVERSTORY COMMUNITY COMPOSITION

Chapter 2: Dominant overstory tree species (> 10 cm DBH) present within 50 m of the center of each plot pair at Dixon Springs State Park (DSSP) and Giant City State Park (GCSP).

Site	Plot Pair	Species Name	Total Species Basal Area (m^2)	Relative Basal Area (spp. BA/total plot BA) (%)
DSSP	I	*Ulmus spp.*	0.64	36.80
		Acer rubrum	0.58	33.36
		Juniperus virginiana	0.29	16.42
DSSP	II	*Fraxinus spp.*	0.48	25.79
		Acer rubrum	0.45	24.42
		Ulmus alata	0.21	11.27
DSSP	III	*Prunus serotina*	0.41	29.30
		Acer saccharum	0.38	27.34
		Ulmus spp.	0.28	20.04
DSSP	IV	*Ulmus spp.*	0.60	46.46
		Juglans nigra	0.16	12.16
		Acer saccharum	0.11	8.68
DSSP	V	*Ulmus spp.*	0.57	42.44
		Juglans nigra	0.38	28.73
		Diospyros virginiana	0.15	11.30
DSSP	VI	*Juglans nigra*	0.99	33.58
		Acer saccharum	0.87	29.45
		Prunus serotina	0.28	9.55
DSSP	VII	*Juglans nigra*	1.27	48.60
		Ulmus spp.	0.79	30.24
		Acer saccharum	0.25	9.58
DSSP	VIII	*Acer rubrum*	1.05	46.35
		Juniperus virginiana	0.28	12.49
		Acer saccharum	0.24	10.81
DSSP	IX	*Fraxinus spp.*	0.46	49.34
		Acer rubrum	0.22	24.21
		Juniperus virginiana	0.13	14.20
DSSP	X	*Fraxinus spp.*	1.22	83.81
		Sassafras albidum	0.22	14.91
		Diospyros virginiana	0.02	1.28
DSSP	XI	*Juglans nigra*	0.36	26.59
		Fraxinus spp.	0.34	25.47
		Acer saccharum	0.34	25.03
DSSP	XII	*Fraxinus spp.*	0.46	56.08
		Ulmus spp.	0.15	18.02
		Diospyros virginiana	0.12	13.94
DSSP	XIII	*Juniperus virginiana*	0.71	45.26
		Ulmus spp.	0.51	32.89
		Diospyros virginiana	0.18	11.22
DSSP	XIV	*Ulmus spp.*	0.38	35.91
		Acer rubrum	0.23	21.85
		Sassafras albidum	0.21	19.73
GCSP	XV	*Acer negundo*	0.64	79.30
		Ulmus spp.	0.10	12.70
		Juglans nigra	0.04	4.70

GCSP	XVI	*Quercus imbricaria*	0.49	23.11
		Ulmus spp.	0.39	18.58
		Quercus rubra	0.25	12.09
GCSP	XVII	*Platanus occidentalis*	1.94	52.67
		Juglans nigra	0.60	16.24
		Liriodendron Tulipfera	0.42	11.42
GCSP	XVIII	*Acer negundo*	0.42	32.59
		Prunus serotina	0.17	13.08
		Quercus rubra	0.17	13.39
GCSP	XIX	*Ulmus spp.*	0.64	39.85
		Juglans nigra	0.35	22.02
		Acer negundo	0.24	15.10
GCSP	XX	*Quercus alba*	0.76	37.62
		Quercus imbricaria	0.63	30.88
		Ulmus spp.	0.24	11.83

Chapter 3: Dominant overstory tree species (> 10 cm DBH) present within 50 m of the center of each plot pair at Dixon Springs State Park (DSSP) and Giant City State Park (GCSP).

Site	Plot Pair	Species Name	Total Species Basal Area (m^2)	Relative Basal Area (spp. BA/total plot BA) (%)
DSSP	V	*Ulmus spp.*	0.57	42.44
		Juglans nigra	0.38	28.73
		Diospyros virginiana	0.15	11.30
DSSP	VIII	*Acer rubrum*	1.05	46.35
		Juniperus virginiana	0.28	12.49
		Acer saccharum	0.24	10.81
DSSP	X	*Fraxinus spp.*	1.22	83.81
		Sassafras albidum	0.22	14.91
		Diospyros virginiana	0.02	1.28
DSSP	XI	*Juglans nigra*	0.36	26.59
		Fraxinus spp.	0.34	25.47
		Acer saccharum	0.34	25.03
DSSP	XII	*Fraxinus spp.*	0.46	56.08
		Ulmus spp.	0.15	18.02
		Diospyros virginiana	0.12	13.94
DSSP	XIII	*Juniperus virginiana*	0.71	45.26
		Ulmus spp.	0.51	32.89
		Diospyros virginiana	0.18	11.22
DSSP	XIV	*Ulmus spp.*	0.38	35.91
		Acer rubrum	0.23	21.85
		Sassafras albidum	0.21	19.73
GCSP	XVI	*Quercus imbricaria*	0.49	23.11
		Ulmus spp.	0.39	18.58
		Quercus rubra	0.25	12.09
GCSP	XVII	*Platanus occidentalis*	1.94	52.67
		Juglans nigra	0.60	16.24
		Liriodendron Tulipfera	0.42	11.42
GCSP	XVIII	*Acer negundo*	0.42	32.59
		Prunus serotina	0.17	13.08
		Quercus rubra	0.17	13.39
GCSP	XIX	*Ulmus spp.*	0.64	39.85
		Juglans nigra	0.35	22.02
		Acer negundo	0.24	15.10
GCSP	XX	*Quercus alba*	0.76	37.62
		Quercus imbricaria	0.63	30.88
		Ulmus spp.	0.24	11.83

APPENDIX C: SOIL RESPIRATION MODELING COEFFICIENTS

Chapter 2: Beta coefficients derived for each plot used to predict hourly soil respiration using a log-linear quadratic model based on hourly soil temperature (*soilT*) and soil moisture (*SWC*).

Site	Plot Pair	Treatment	b_0 *(intercept)*	b_1 *(SWC)*	b_2 *(SWC^2)*	b_3 *(soilT)*	b_4 *(soilT^2)*	b_5 *(SWC * soilT)*
DSSP	I	burn	-4.0207	14.7761	-19.6982	0.2422	-0.0021	-0.1944
DSSP	I	ambient	-7.6498	22.0616	-19.3187	0.4916	-0.0054	-0.5225
DSSP	II	burn	-1.8163	1.3538	-5.4462	0.2013	-0.0034	0.1446
DSSP	II	ambient	0.1272	0.4371	-15.6965	-0.0046	0.0009	0.4338
DSSP	III	burn	4.8964	-16.6509	0.5154	-0.2394	0.0033	0.8610
DSSP	III	ambient	-3.0494	6.2559	-6.2991	0.2113	-0.0012	-0.0651
DSSP	IV	burn	1.3742	-4.3794	-7.0254	-0.0388	0.0011	0.4171
DSSP	IV	ambient	-2.8550	4.8081	-5.0783	0.2520	-0.0037	-0.0167
DSSP	V	burn	1.3391	-3.4662	-4.0668	-0.0583	0.0019	0.3588
DSSP	V	ambient	2.4376	-9.4166	0.9831	-0.1025	0.0018	0.4867
DSSP	VI	burn	2.4913	-13.7586	-2.3868	-0.0412	0.0000	0.6965
DSSP	VI	ambient	-4.0493	5.0040	-6.7313	0.3832	-0.0068	-0.0136
DSSP	VII	burn	2.0076	-5.9264	-6.5686	-0.0310	-0.0007	0.4829
DSSP	VII	ambient	4.4099	-13.1933	1.7622	-0.2291	0.0036	0.6432
DSSP	VIII	burn	-0.4093	0.0319	-10.7989	0.1154	-0.0027	0.2906
DSSP	VIII	ambient	-3.1095	9.2378	-14.8165	0.2204	-0.0022	-0.0565
DSSP	IX	burn	-1.7523	4.8942	-8.9232	0.1825	-0.0022	0.0248
DSSP	IX	ambient	-0.6273	-2.6746	-0.5902	0.1699	-0.0033	0.2190
DSSP	X	burn	-2.5770	3.1634	1.5302	0.2522	-0.0030	-0.0767
DSSP	X	ambient	-3.2381	4.6653	-1.9473	0.3126	-0.0047	-0.0989
DSSP	XI	burn	-2.1366	3.6391	-9.9744	0.2279	-0.0043	0.1399
DSSP	XI	ambient	-5.4553	15.7053	-22.5541	0.4532	-0.0087	-0.1974
DSSP	XII	burn	-8.7694	20.5117	-5.5835	0.6542	-0.0098	-0.7114
DSSP	XII	ambient	-11.0353	22.5350	1.1972	0.9409	-0.0169	-0.9673
DSSP	XIII	burn	-7.4191	22.6002	-12.4568	0.4877	-0.0050	-0.7108
DSSP	XIII	ambient	-2.6938	3.8595	-5.0785	0.2201	-0.0032	0.0093
DSSP	XIV	burn	-4.4818	15.2020	-11.0506	0.2627	-0.0016	-0.4092
DSSP	XIV	ambient	-11.7435	26.3624	-5.4903	0.8555	-0.0124	-1.0542
GCSP	XV	burn	-1.1097	-0.1259	-16.4257	0.1197	-0.0041	0.5142
GCSP	XV	ambient	0.7470	-0.6126	-14.5145	-0.0482	0.0008	0.4797
GCSP	XVI	burn	-0.7865	4.5781	-11.5383	0.0266	0.0014	0.1334
GCSP	XVI	ambient	0.9719	-0.2507	-8.6646	-0.0733	0.0031	0.3164
GCSP	XVII	burn	2.6762	-8.4153	14.2356	-0.1470	0.0087	-0.1493
GCSP	XVII	ambient	3.5415	4.6648	-28.4472	-0.3672	0.0084	0.5636
GCSP	XVIII	burn	1.7170	1.1403	-8.8691	-0.1773	0.0069	0.2146
GCSP	XVIII	ambient	1.2365	-0.4384	-13.6300	-0.0634	0.0019	0.4473
GCSP	XIX	burn	2.3386	-3.2567	-15.0339	-0.1757	0.0029	0.5962
GCSP	XIX	ambient	3.9174	-3.1945	-22.4960	-0.2931	0.0052	0.7615
GCSP	XX	burn	-2.5284	4.8802	-11.9781	0.2860	-0.0060	0.1356
GCSP	XX	ambient	-2.9254	9.1998	-26.1561	0.3213	-0.0066	0.0664

Chapter 3: Beta coefficients derived for each plot used to predict hourly soil respiration using a log-linear quadratic model based on hourly soil temperature (*soilT*) and soil moisture (*SWC*).

Site	*Plot Pair*	*Treatment*	b_0 *(intercept)*	b_1 *(SWC)*	b_2 *(SWC^2)*	b_3 *(soilT)*	b_4 *(soilT^2)*	b_5 *(SWC * soilT)*
DSSP	V	ambient	2.4376	-9.4166	0.9831	-0.1025	0.0018	0.4867
DSSP	V	drought	0.7255	-2.1276	-12.2683	0.0468	-0.0028	0.3754
DSSP	VIII	ambient	-3.1095	9.2378	-14.8165	0.2204	-0.0022	-0.0565
DSSP	VIII	drought	8.5387	-0.3429	-26.8608	-0.8880	0.0219	0.7294
DSSP	X	ambient	-3.2381	4.6653	-1.9473	0.3126	-0.0047	-0.0989
DSSP	X	drought	-27.3087	61.8591	-47.7111	2.3328	-0.0492	-1.8756
DSSP	XI	ambient	-5.4553	15.7053	-22.5541	0.4532	-0.0087	-0.1974
DSSP	XI	drought	-22.8559	67.1640	-90.3499	2.1857	-0.0540	-1.6656
DSSP	XII	ambient	-11.0353	22.5350	1.1972	0.9409	-0.0169	-0.9673
DSSP	XII	drought	-7.4703	32.2245	-28.7414	0.4903	-0.0074	-0.8134
DSSP	XIII	ambient	-2.6938	3.8595	-5.0785	0.2201	-0.0032	0.0093
DSSP	XIII	drought	-8.6912	23.1844	-17.8828	0.5703	-0.0083	-0.5861
DSSP	XIV	ambient	-11.7435	26.3624	-5.4903	0.8555	-0.0124	-1.0542
DSSP	XIV	drought	-11.9949	34.0415	-16.7913	0.8679	-0.0133	-1.1588
GCSP	XVI	ambient	0.9719	-0.2507	-8.6646	-0.0733	0.0031	0.3164
GCSP	XVI	drought	13.3614	-20.9670	-2.7491	-1.3095	0.0325	1.2497
GCSP	XVII	ambient	3.5415	4.6648	-28.4472	-0.3672	0.0084	0.5636
GCSP	XVII	drought	-1.5928	14.0851	-17.7098	0.0867	0.0025	-0.3584
GCSP	XVIII	ambient	1.2365	-0.4384	-13.6300	-0.0634	0.0019	0.4473
GCSP	XVIII	drought	7.4827	-35.6389	-2.1698	-0.6165	0.0137	1.9891
GCSP	XIX	ambient	3.9174	-3.1945	-22.4960	-0.2931	0.0052	0.7615
GCSP	XIX	drought	-5.1075	13.7441	-21.3415	0.5270	-0.0122	-0.1560
GCSP	XX	ambient	-2.9254	9.1998	-26.1561	0.3213	-0.0066	0.0664
GCSP	XX	drought	3.8078	-0.3183	-24.5785	-0.3679	0.0103	0.5669